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EVIDENCE OF VASCULAR PLANTS IN THE CAMBRIAN

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During the last two decades attention has been focused on spore discoveries in Early Palaeozoic rocks, and several varied types of spores have been distinguished. While the information given by palynology agrees with the flora known in Upper and Middle Devonian, the assemblage of spores found in Lower Devonian, Silurian and Cambrian plead in favor of a more varied and advanced type of flora than the plant impressions have so far suggested. The recent discovery of Lycopodiaceous shoots in the Middle Cambrian of East-Siberia appears to confirm the evidence of palynology investigation. The purpose of this article is to emphasize the meaning of these discoveries and to discuss briefly their bearing on questions of evolution.

Our knowledge of the Devonian flora has increased importantly during the last three decades, largely as a result of the research carried out in Canada, the United States, Bohemia, Great Britain, Germany, Norway, Sweden, and Belgium. One of the most striking features is the sharp difference which appears to exist between the flora of the three main divisions of the Devonian.

As is well known, the Upper Devonian landscape foreshadows the Coal period scenery. The same groups of pteridophytes are represented by identical and distinct genera. Preserved structures of primitive gymnosperms are also known. Some of these plants are tree-forms, with large fronds and well developed leaves. This is confirmed by palynology. Spores and pollen grains found in Upper Devonian strata show a great variety of pattern, size, and ornamentation (Naumova, 1953; Radforth and McGregor, 1954), which display affinity with filicinean, calamarian, and lycopodiaceous spores, and pollen grains of Pteridospermales, Cordaitales and Coniferales.

In the Middle Devonian, the cover of vegetation is peculiar. While members of the main races of the pteridophytes and some primitive woods of Gymnosperms are represented, most of the genera are different and simplified in habit. That is to say: though the plants show an average level of high differentiation, most of them are not tree-form, and have only minute leaves. Moreover, a few groups exist which, hitherto, have appeared to have developed during Middle Devonian: the *Proto-Articulatae,* the *Iridopteridaceae,* the *Cladoxylaceae,* etc. In a parallel way, researches on microfossils bring out a diversity of types. These, however, show less complexity of pattern than do the spores and pollen grains of the Upper Devonian (Thomson, 1952; Radforth

and McGregor, 1954). Yet, forms and types resembling filicinean, calamarian and lycopodiaceous spores have been discovered.

The vegetation in the Lower Devonian offers a sharp contrast with that of the Middle Devonian, on the basis of the record of impression so far collected. The Lower Devonian flora cover would appear to have consisted of a carpeting of slender, tufted, herbaceous and semi-aquatic plants, mostly consisting of Psilophytales or psilophyte-like forms, with a few lepidodendroid genera like *Drepanophycus* and *Protolepidodendron*. The marked discrepancy observed between two floras so close in geological time as Middle and Lower Devonian, is difficult to reconcile. The absence in Lower Devonian of plant impressions positively related to any of the varied differentiated plants of the Middle Devonian is astonishing. On the other hand, the present difficulty concerning the characterization of the psilophytalean complex leads one to speculate that the latter might possibly include representative members of other phyla, in which the external morphology may have been very similar as a consequence of an adaptation to a similar set of life conditions (Leclercq, 1954).

Personal field work during the last summer has shown that the main genera of the Middle Devonian flora occur at the very beginning of the period (base of the Couvinian). So far the best known exposures are located in the upper part of the Middle Devonian (Givetian). It is now confirmed that the same differentiated flora existed through the whole sequence (Liégeois, 1953, 1955). On the other hand, in the subdivision immediately below, the Emsian (upper part of the Early Devonian), occur the monotonous undifferentiated plants of the Early Devonian. These facts provide a basis for inference that more specialized plants may have existed in Lower Devonian, simultaneously with the psilophytalean complex and lycopsids, which probably represented the dominant ecological facies of the period. Contemporary with these, however, the precursors of the continental races which flourished in Middle Devonian may have colonized the xerophytic habitat of the Lower Devonian. Relics of these precursors have yet to be found. Field work devoted to a search for synchronous bio-facies in Lower and Middle Devonian strata is being continued in Belgium. A few examples have already been located.

The results obtained in Early Devonian palynology are extremely interesting. Thomson, in 1952, emphasized the distinctions observed between spore assemblages in Lower Devonian of Germany and Middle Devonian of Esthonia. His observations are in accord with the evidence from the megaflora hitherto collected; i.e., spores found in Middle Devonian show complex morphological features while spores that are primitive in shape and ornamentation are present in Lower Devonian (Lang, 1925; Höeg, 1942). More recently, however, Radforth and McGregor investigated sediments in Canada. They write (1954, p. 615): "Spore complexity is also a feature of floras of the Lower Devonian and Silurian periods, as indicated by the microfossils of Figs. 57 to 74. Prominence of complexity (pattern and form) is on the other hand less obvious than for the Middle and Upper Devonian rocks." The observations of Thomson, although they appear opposed to those of Radforth and McGregor, are in fact complementary. Moreover, they strengthen the suggestion that various bio-facies may have existed during the Lower Devonian, and even suggest similar conditions in Silurian.

The Siluro-Devonian of Australia has yielded compressions of the oldest vascular plants so far collected. These remains reveal plants of different types, habits and habitats. Besides the *Yarravia* and *Hedeia* of uncertain systematic position, there are representative members of the psilophytales-complex and the differentiated *Baragwanathia* (Lang and Cookson, 1935). The occurrence of two phyla: Psilophytales and Lycopsida in Siluro-

Devonian suggests the possible co-existence of other races of vascular plants in that period (Leclercq, 1954). As has been noted, this suggestion receives the support of palynology (Radforth and McGregor, 1954).

The Cambrian megaflora has until recently yielded no evidence, although palynology has argued for the existence of varied vascular plants. According to Reissinger (1939) and Naumova (1949), the spores found in the blue-clay of Lower Cambrian of Kunda in Esthonia and of Pre-Baltique in U.S.S.R. are cutinized; generally very small in size; 15 up to 27 microns (exceptionally 75 microns); regular in shape, round or oval; and smooth or slightly grained, irregularly multifolded, with triradiate marking more or less distinct. In greater contrast are the spores found in the Upper and Middle Cambrian of Kashmir, Spiti, and the Neobolus shale from Salt Range in India. If the majority of these are round or oval in shape, triangular forms are not uncommon (Jacob, 1953). A coarse network ornamentation, continuous membranous wing and even bladders (?) are observed (Ghosh and Bose, 1950; Ghosh, Sen and Bose, 1951; Ghosh and Bose, 1952; Jacob, 1953). The size of the spores generally varies from 5 to 50 microns with the largest ones ranging from 25 to 130 microns. Most of the samples are geologically authenticated by the characteristic fauna (trilobites and brachiopods) of the horizons (Jacob, 1953 a and b). So far, at least, fifty types of spores have been distinguished.

Such a remarkable variety of spores in Cambrian sediments is impressive and certainly unexpected. Moreover, besides the spores Reissinger (1939), Ghosh and Bose (1950), Ghosh, Sen, Bose (1951), Ghosh and Bose (1952), and Jacob (1953 a–b) have found tiny fragments of woody elements, which, despite poor preservation, show scalariform and pitted (simple and bordered pits) tracheids.

The conclusion to be drawn from these facts was that varied types of vascular plants existed in Early Palaeozoic. However, scepticism was prevalent among scientists. It was suspected that the samples of rocks were probably contaminated by younger sediment. That is why Indian workers repeatedly checked their results, and this always with success. Nevertheless the persistent lack of floral remains in Cambrian came into conflict with palynology. Furthermore, the existence of such a simple group of vascular plants as the Psilophytales in Upper Silurian supported the hypothesis that this may have represented the starting point of terrestrial plant life. Fortunately, the discrepancy between micro-flora and mega-flora is now in process of vanishing.

In the Middle Cambrian of East Siberia a lycopodiaceous plant has been discovered (Kryschtofowitch, 1953). The geological age of the deposit is determined by the following fauna: *Prohedinia attenuata* Lerm, *Ptychoparia tchabdensis* N. Tchern, *Anomocariodes limbataeformis* Lerm and *Hyagnostus truncacus* (Brögg). According to the author this fauna corresponds in age to the West European zone with *Paradoxides forchammeri* from the Middle Cambrian. He writes as follows:

> "The plant material consists of 4 impressions of shoots, the fragments of which are 4 up to 8.5 cm. long, and 8 to 13 mm. broad. The surface of the axe is covered fairly evenly with oblong thickenings in regular arrangement. The thickenings are extended upwards and downwards by thin stripes giving the whole shoot a wrinkled appearance. In addition the surface of the shoot is minutely granulated. The shoot is densely covered with thin and delicate appendages (leaves) which are up to 9 mm. long. On the impressions the edge of the shoot is uneven, as if dentate due to the form of the thickenings and the arrangement of the appendages. In some places it is possible to trace a thin stripe, a vascular bundle right out to the base of the thickenings. . . ." . . . "Attempts to isolate the cuticule or epidermis through mazeration or to obtain a collodion impression have not been successful."

Kryschtofowitch points out that the Cambrian plant differs very much from the typical psilophytes (*Psilophyton, Rhynia, Hornea, Asteroxylon*) and may rather

be compared with *Drepanophycus* and *Baragwanathia*. He says: "In its sculpture our plant very much resembles *Drepanophycus spinaeformis;* in the latter, however, the appendages look like thorns, while appendages (leaves) in *Baragwanathia* very much resemble those of *Aldanophyton antiquissimum*" (the new plant name). Consequently Kryschtofowitch comes to the conclusion that the Cambrian plant ". . . seems rather to show that it belongs to the primitive lycopodiales, not to the psilophytes." In accordance with the lack of sporangia and anatomy, the author separates the new plant under a new genus which, he says, may be a provisional name.

Kryschtofowitch notes the findings, in the Cambrian, of a land plant, probably belonging to the lycopodiales, and various kinds of spores, and points out "That these discoveries throw a new light on the problem of the origin of land plants and permit us to trace higher cryptogames still further back in time."

While the affinity of *Aldanophyton* to the Lycopodiales can hardly be questioned, the figured specimens suggest a morphology more related to the genus *Drepanophycus* than to *Baragwanathia*. In fact there are some species within the genus *Drepanophycus* on which the appendages are more leaf-like than thorn-like. On the other hand, the form and arrangement of the thickenings on the surface of *Aldanophyton* appear to be closely comparable, in the essential features of their construction, to those of *Drepanophycus*. In view of the fragmentary condition in which the specimens are preserved, a generic discussion is not pertinent to this report.

Because the discoveries of differentiated spores and a vascular plant, in Cambrian, may be considered as among the the most interesting results of recent palaeobotanical records, we feel justified in commenting briefly on their significance.

The remarkable fact is that a lycopsid more complex in structure than the Psilophytales has been found in a horizon definitely earlier than any from which the latter is known. It positively separates the origin of the lycopsids from the Psilophytales. Furthermore, the degree of differentiation exhibited by *Aldanophyton* presupposes a long evolutionary sequence prior the Cambrian. Within the limits of the lycopsids it shows the persistency of a *Drepanophycus*-like type from, at least, the Middle Cambrian to the base of Middle Devonian without much modification; that is to say, during a period of about two hundred million of years. In addition there arises the question of a probable co-existence of other phyla of vascular plants in Cambrian and even earlier. Furthermore, it accredits the Cambrian spores and their conclusion.

It is of great interest that the plant-microfossil and the megaflora show close agreement, for, in that way, the credit of palynology to predict the complexity of a flora is strengthened. Spores and pollen grains probably give a fairly adequate expression of the components of the vegetation which were flourishing during a geological period, for, by reason of their capacity to disperse, they may convey information as to types of plants which owing to their habitats have little chance to be preserved. Continental formations are generally unsuitable for preservation of plant impressions; land and fresh water deposits are peculiarly liable to destruction. These circumstances, for example, are responsible for the scarcity of our documentation on the upland plants and the fragile Bryophyta elements within the flourishing Carboniferous flora.

Similarly, the accordance of the results of palynology and plant impressions through the Early Paleozoic adds convincing support to the usefulness of spore applications for zonal analysis in economical purposes: study of stratigraphy, petroleum geology, etc.

Incidentally palynology focuses attention on the need for caution in the interpretation of ecological facies of sedimentary rocks. On the basis of a megaflora,

ecological associations may erroneously be considered as evolutionary stages. For instance the *Rhyniaceae,* upon which most of our information about the structure of the Lower Devonian plants is based, owe the simplicity of their structure *in part* to the peculiar environment to which they are adapted. Though primitive in features, they probably represent relic forms in a plant world composed of varied and more highly organized forms whose complexity we only are beginning to foresee (Leclercq, 1954). The variety of spores found in Siluro-Devonian (Radforth and McGregor, 1954) reinforces this opinion as it indicates that a vegetation more differentiated than the Psilophytales had taken possession of the land at that time.

In a wider sense palynology and plant impressions of Cambrian raise the major question of the polyphyletism of the vascular plants. They carry back to Proterozoic the fundamental preliminary process that have prepared for the differentiation of the main races, which, later, have given rise to a land flora. They indicate a more remote time for the epoch of the migration from sea to land, hitherto generally considered to have occurred during Siluro-Devonian. Moreover, the assumption that a varied flora existed in Cambrian time should adjust the flora to faunas which, in Lower Cambrian, show a burst of divers creative activity.

It is important to bear in mind that algal structures have been detected in graphitic limestones of 3000 million of years (1941, A. M. Macgregor). The alga belongs to the Stromatolites and shows well preserved graphitic, concentric laminae. The limestone belongs to the Bulawayan System in Southern Rhodesia; its age has been established by the analysis of monazites, which indicates an age of some 2650 million years (1955, A. Holmes and L. Cahen). Thus the threshold of the sedimentary rocks is crossed opening up a tremendously large field of research. If Stromatolites represent organic remains, then the enormous duration of time available for the development of distinct, unknown ancestors throws stimulating new light on the potentialities of the original differentiation of the Plant Kingdom.

Persistence of type with imperceptible change and, from time to time, the sudden influx of new types, correlative with favorable stable geological conditions, are among the outstanding features of the history of evolution as shown by palaeontology.

On the contrary periods of profound change in fossil floras are observed in relation to major geological events as: orogenic movements, great marine transgressions, glaciations, important changes of climatic equilibrium, etc. All these perturbations must have had a far reaching influence on the evolution of plant world.

Thus the knowledge gained from the investigation of the older formations changes our perspective on the preliminary processes of plant differentiation on the earth and provokes thought and research.

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THE GENETICS AND EVOLUTION OF GYNODIOECY

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Introduction

To know the breeding system is to know the genetic architecture of a species. To know the evolution of a breeding system is to know how evolution works—on populations and not on individuals. One of the many different breeding systems in plants is male sterility which has been found as a rare mutant in all higher plants that have received more than a cursory genetical examination. A recessive gene is the most common genetical control, but there are several examples of cytoplasmic determination.

In contrast to this rare type of male sterility, there are many species, particularly in the Labiatae, which have a high proportion of male sterile individuals in the population. Such species are described as **gynodioecious**, and consist of hermaphrodites and females. Several species were described by Darwin (1877) who made observations on the progeny and the relative fertilities of the two types. Correns (1928 and earlier) studied the inheritance of the sexual difference and found that in *Satureia hortensis* and *Circium oleraceum*, the females and hermaphrodites both bred true. He also carried out backcrossing for many generations until all the nuclear genes would be from the pollen (i.e. ♀) parent, but despite this the female condition still persisted and bred true. This was interpreted on a cytoplasmic basis and has now become one of the classical examples of a character under the control of a permanent cytoplasmic particle or plasmagene. East (1934), however, gave a possible alternative explanation which did not invoke the cytoplasm.

In many other gynodioecious species Correns found that the two types, female and hermaphrodite, segregated both types in their progeny. No genetical explanation has been found for these segregating species.

Gynodioecy constitutes an outbreeding system, for the hermaphrodites may be self-pollinated but the females must be cross-pollinated. With such a system varying degrees of outbreeding can be obtained according to the proportion of females in the population. It differs from all other outbreeding mechanisms such as incompatibility and dioecy, in that the two mating types contribute unequal numbers of nuclear genes to the next generation. Plant for plant a hermaphrodite contributes three times as many genes as does a female, for it supplies its own pollen and egg nuclei and also the pollen nucleus for the female. The theoretical consequences of this for male sterility under the control of a single recessive or dominant gene is, that if females are to occur more frequently in the population than the mutation rate, then they must have at least a two-fold initial selective advantage over the hermaphrodites. If, however, male sterility is controlled by the cytoplasm and the pollen passes no cytoplasm to the zygote, or the little that does pass is quickly suppressed by the larger amount of cytoplasm supplied by the embryo-sac, then the hermaphrodite and female will contribute equal amounts of the all-important sex determiners which are in the cytoplasm to the next generation. With cytoplasmic control, therefore, an equilibrium similar to the equality of the sexes in a dioecious species could be obtained without a two-fold selective advantage of females over hermaphrodites (Lewis, 1941).

A study of females and hermaphrodites in *Origanum vulgare* was undertaken in the hope that it might clarify the meaning and evolution of this unusual but not uncommon type of outbreeding mechanism.

Material

Origanum vulgare is a Labiate with a high proportion of females in its populations. We have seen them in populations growing in a wide variety of environments in England, France, Sweden, Germany, Austria, Switzerland, and Italy. Their proportion cannot be given accurately because of vegetative reproduction, but it would appear to be at least 30% and is more probably 50%.

The original parents of all progeny were collected in 1947 from a wild population growing on the North Sussex Downs at Berry Hill, England. Three hermaphrodites and three females were dug up while in bloom, potted and bred from in the same year.

Results of Breeding Tests

In the first year the wild hermaphrodites were selfed and crossed on to the three females. All the hermaphrodites bred true and all but one small family of the crosses between females and hermaphrodites segregated both types as shown in table 1.

Table 1. *Progeny obtained from selfing hermaphrodites (3), (5), (6), (7) and from crossing these on to females (1), (2) and (4): all plants collected from the wild*

	1st Gen. ♀	Selfing ☿	2nd Gen. ♀	Selfing ☿
☿ (3)	0	187		
☿ (5)	0	132	0	120
☿ (6)	0	32		
☿ (7)	0	5		
	0	356	0	120

	♀	☿
♀ (1) × ☿ (5)	16	39
♀ (2) × ☿ (5)	156	209
♀ (2) × ☿ (6)	27	40
♀ (2) × ☿ (3)	28	46
♀ (4) × ☿ (3)	0	5
	227	339

The wild hermaphrodites were true breeding, and this is in agreement with a cytoplasmic control, but this explanation is ruled out by the segregating families from the crosses between females and hermaphrodites. These crossed families derived from different females and hermaphrodites appear to be homogeneous (heterogeneity X^2 for 4 D.F. is 7.3217, $p = .20–.10$). Furthermore most of the small amount of heterogeneity which is present comes from the small family ♀ (4) × ☿ (3) with only five plants which are all hermaphrodite. The female of this family has not been tested further, and it is possible that it may be different from the others. We can conclude therefore that there is no evidence from these crosses that either females 1 and 2 and/or ☿s 3, 5 and 6 differ within themselves.

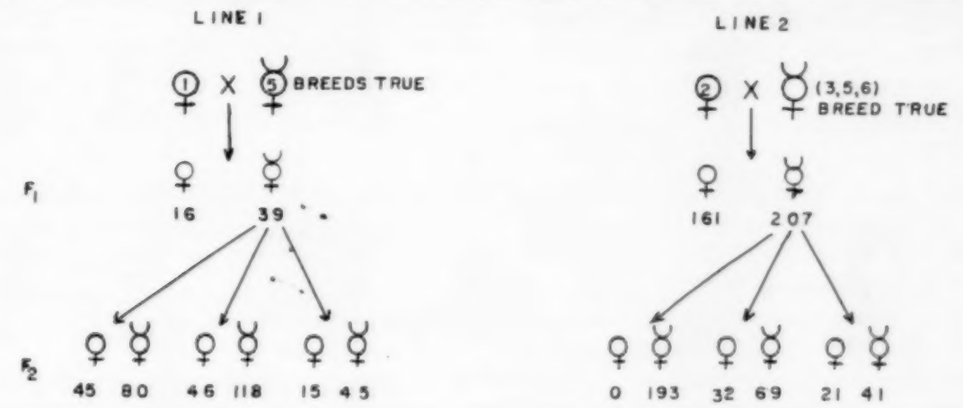
The total segregation of 227 ♀ to 339 ☿ is not any normal ratio: it could be a disturbed 1:1 or 3:1 ratio.

Two of the crosses ♀ (1) × ☿ (5) (line 1) and ♀ (2) × ☿ (5) (line 2) were selected for further breeding. The results of two generations of breeding with these two lines are given in table 2.

There is clearly more than one hermaphrodite genotype. The parents are true breeding, but the hermaphrodites in the F_1 generation are of two kinds, true breeding and segregating. The five segregating F_2 families give a total of 159 ♀ and 353 ☿, which differs significantly from a 3:1, or in fact from any normal ratio, but the families are homogeneous, showing that the deviation has a common cause.

These results at first sight appear paradoxical: for on a single-gene basis the original hermaphrodites, because they do not segregate, would appear to be homozygous. On this interpretation the segregation in the F_1 must mean that the females are heterozygous for a dominant gene, and that the hermaphrodites are

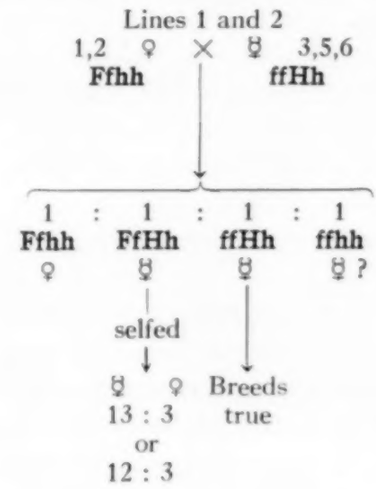
TABLE 2. *Progeny obtained in the* F_1 *and* F_2 *in two lines. Note that despite the fact that the original hermaphrodites breed true, the* F_1 *segregates both types, and that some of the* F_1 *hermaphrodites breed true and others segregate in the* F_2 *generation.*



recessive homozygotes. But clearly this is not so because of the segregating hermaphrodites in the F_2.

The interpretation by which these families and the whole of the data will be explained is based upon two independent genes. A dominant gene, **F**, which causes the anthers to abort, and a dominant gene, **H**, which suppresses the action of **F**. Thus a female can be either **FFhh** or **Ffhh**, both of which will give segregating families when crossed with hermaphrodites; while a hermaphrodite may be **ffHh** which will breed true on selfing and yet give a segregating family when crossed on to females, and **FfHh** which will segregate on selfing and on crossing on to females. This is the only interpretation possible on a disomic basis, and it is given now before all the data have been presented, for ease of description. But since *Origanum vulgare* has 32 chromosomes with n = 8 it may have a tetrasomic complement, and therefore other possible interpretations on a tetrasomic basis are described and rejected in the discussion.

TABLE 3. *Genetic constitution of plants and expected progeny in lines 1 and 2, based on a dominant gene* **F** *which causes anther abortion and* **H** *which is a dominant suppressor of* **F**



In table 3 the interpretation based upon disomic inheritance of the two genes **F** and **H** is shown for the two generations represented in table 2.

In the first generation the expected ratio is 3:1 if the bottom recessive is viable and is a hermaphrodite, and 2:1 if it is lethal. The figures do not fit a 3:1 ratio but with one exception the X_2 for a 2:1 ratio is well below that at the .05% level of significance. In the second generation, the expected ratio in the segregating families is 13:3 if the bottom recessive is viable or 12:3 if lethal. The observed figures deviate significantly from both these ratios, and in all the families the females are in excess of expectation. But these bad ratios do not vitiate the most important and critical agreement with the two-gene hypothesis,

TABLE 4. *Results of selfing and crossing hermaphrodites and females which have been selected from segregating* F_2 *families*

Fam.	Line 1 ♀ × ☿		☿ selfed, progeny ♀	☿	crossed, progeny ♀	☿
1	**Ffhh**	**FfHh**	21	80	16	24
2	**FFhh**	**FfHh**	2	11	40	40
3	**Ffhh** ?	**FfHh** ?	—		36	80
4	**Ffhh** ?	**FfHh** ?	—		12	36
5	**Ffhh**	**ffHh**	0	161	62	116
	Line 2					
6	**FFhh**	**FfHh**	24	88	116	119
7	**Ffhh**	**FfHh**	21	95	35	134
8	**Ffhh**	**FfHh**	—		36	75
9	**Ffhh**	? **HH**	0	7	0	58

i.e. the unusual distribution of uniform and segregating families in the selfed, F_1 and F_2 generations.

The data for the third generation are given in table 4. Four of the selfed families segregate and two breed true. The small number of seven plants in one of these families is not conclusive evidence for a true breeding parent, but the absence of females in the larger cross family from this hermaphrodite proves its genetic constitution to be **HH**. The lack of selfed progeny from this plant is due to self sterility. The plant was fully fertile in open pollination but on selfing many spikes of flowers in two consecutive years, only eight seeds were produced.

The expected proportions of different hermaphrodites in the second generation are:

1 **FFHH**	2 **FFHh**
2 **FfHH**	4 **FfHh**
1 **ffHH**	2 **ffHh**

All **HH** and **ff** types will breed true, making an expected ratio of one true breeder to one segregating. The observed numbers of two true breeders to four segregating do not differ significantly from expectation.

In the crosses, two families give equality of females to hermaphrodites thus showing that the female parent is homozygous **FFhh**. The observed figures for identified female genotypes are seven **Ffhh** to two **FFhh** which is in good agreement with the expected 2:1 ratio.

Several families were carried on to the fourth generation to substantiate the theory of two genes and to give evidence on points such as the disturbed ratios and the possible inviability of some genotypes. Fifteen families were raised to the fourth generation and no exceptions apart from the deviations from the expected ratios were found. Only certain of these families of special interest will be described.

Three hermaphrodites from the crossed family 2 in table 4 were selfed. All segregated females and hermaphrodites as expected because if the female parent of the family is **FFhh** then all its progeny would carry the gene **F**. Another fourth generation family was obtained by crossing a female with a hermaphrodite from the same family 2. The result was 168 hermaphrodites and 180 females. This 1:1 segregation confirms that the female parent was **FFhh** and the ☿ **FfHh** and, thus proves the existence of **FFhh** females.

Four hermaphrodites from family 1 in table 4 were also selfed. Two of these plants bred true and two segregated, thus showing that the progeny included the hermaphrodite genotype **ffHh** as expected and that the female parent was **Ffhh**.

Lethality and Disturbed Segregation

The results have established that the difference between the hermaphrodite and female is controlled by the major genes **F** and **H**. The disturbed ratios obtained in many families, far from invalidating the conclusions, do in fact have an explanation which is of importance in the evolution of the system, and in maintaining the selective balance between the two types in nature.

In table 5 the figures for the four different types of segregating families are separated into successive generations descended from the wild material. The ratio of hermaphrodites to females may be compared with the expected ratios.

Four points stand out: (1) The **FFhh** × **FfHh** families give good 1:1 ratios in all generations. In these families no

TABLE 5. *Segregation of females and hermaphrodites in families arranged into successive generations descended from the wild material. Note the improvement in the fit to expectation in the later generations*

Generation	**FfHh** selfed	**Ffhh** × **FfHh**	**Ffhh** × **ffHh**	**FFhh** × **FfHh**
1			16 39 28 46 156 209 27 40 227 334 1 : 1.5	
2	21 41 32 69 45 80 15 45 46 118 159 353 1 : 2.2			
3	2 11 21 80 24 88 21 95 68 274 1 : 4.0	35 134 16 24 51 158 1 : 3.1	36 75 62 116 36 80 12 36 146 307 1 : 2.1	109 116 40 40 149 156 1 : 1.0
4	14 90 57 188 71 278 1 : 3.9		2 6 30 89 2 21 31 102 11 18 30 79 106 315 1 : 3.0	168 180 1 : 1.0
Expected without Lethality	1 : 4.3	1 : 1.6	1 : 3	1 : 1
Expected with Lethality	1 : 3	1 : 1.3	1 : 2	1 : 1

TABLE 6. *Identified ☿ s—***F, f** *segregation*

	FfH	ffH	Exp.	Origin of family
F_1 plants	5	1	1:1	(**Ffhh**×**ffHh**)
F_2 plants	4	1	2:1	(**FfHh** selfed)
F_3 plants	2	3	2:1	(**FfHh** selfed)
	H, h *segregation*			
	Hh	**HH**		
F_2 plants	5	1	3:1	(**FfHh** selfed)
F_3 plants	7	0	3:1	(**FfHh** selfed)
	12	1	3:1	

ffhh or **HH** genotypes are expected. (2) In the families, **FfHh** selfed and **Ffhh** × **ffHh**, where either or both of the **ffhh** and **HH** hermaphrodites are expected, there is a deficiency of hermaphrodites. (3) The **FfHh** and **Ffhh** × **ffHh** families in the later generation show an improvement in the ratios due to an increase in the proportion of hermaphrodites. (4) The **Ffhh** × **FfHh** families show an excess of hermaphrodites.

To explain these disturbed ratios we propose to invoke reduced viability or complete lethality of certain genotypes. The bottom recessive **ffhh** has not been identified and we are assuming that it is lethal, at least in the early generations. Other genotypes are produced in reduced numbers. The proportion of genotypes which have been identified from **FfHh** selfed and **Ffhh** × **ffHh** families are particularly instructive. These are given in table 6.

Firstly the figures for **FfH** to **ffH** hermaphrodites in the first two generations show an excess of **FfH** types. In the third generation this excess is not present. The numbers are small and by themselves of little value; but when they are compared with the disturbed ratios in the first two generations and the normal ratio in the third and fourth generations, as shown in table 5, they are significant.

Secondly the figures for **Hh** and **HH** hermaphrodites show that the numbers of **HH** types are significantly lower than expectation.

Assuming from the numbers of identified genotypes that **ffH** hermaphrodites are only 50% viable in the first two generations and that **HH** types are only 25% viable and that **ffhh** are inviable, adjustments to the expected ratios for the first two generations are made below:

	Ffhh × **ffHh** ♀	♂	
Observations	227	334	
Expected	224.4	336.6	2:3

	FfHh selfed ♀	♂	
Observations	159	: 353	
Expected	139.6	: 372.3	3:8

Thus a very satisfactory fit is obtained in the **Ffhh** × **ffHh** families and a reasonable fit with the **FfHh** selfed families.

The only abnormality that is not satisfactorily explained by the above assumption is the excess of hermaphrodite in the **Ffhh** × **FfHh** families. For this we cannot offer an explanation.

The general rise in the number of hermaphrodites in families during four generations in captivity is shown in figure 1. The lethality associated with hermaphrodites and in particular with **ffhh** and **HH** genotypes in wild populations, has been reduced by the artificial conditions imposed by controlled breeding and selection.

FERTILITY

Counts of good seeds from 20 flowers were made on females and hermaphrodites from three families. With four seeds per flower these counts can give a maximum of eighty seeds per plant. From counts on sixty plants the mean percentage of good seed was: ☿ 75% ♀ 89%. This difference is highly significant.

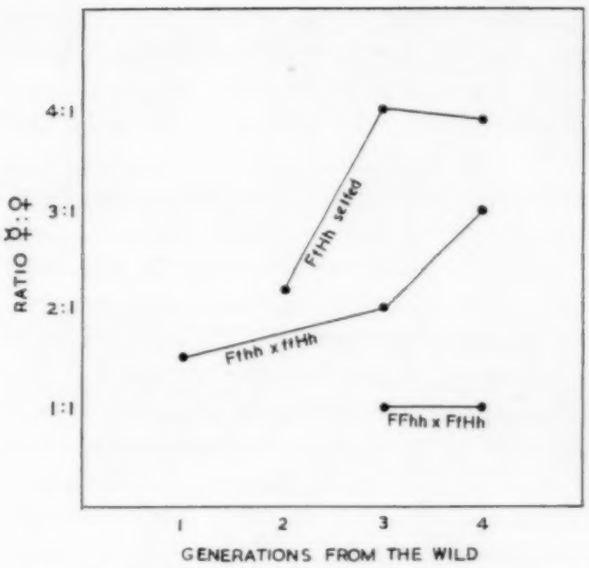


FIG. 1. The general rise in the number of hermaphrodites in families during four generations in captivity.

MUTATION AND CHIMAERAS

Willis (1892) recorded that hermaphrodite plants of *Origanum vulgare* frequently have flowers with aborted anthers and small corollas as in the females. Among the thousands of seedlings grown in our experiments, female flowers and female shoots have been found on hermaphrodite plants. But a hermaphrodite shoot on a female plant has not been found.

Out of the 74 families raised, chimaeras due to a mutation from ☿ → ♀ have been found in only seven. Thirty-three chimaeras were found in these seven families among 632 hermaphrodite plants. All these families had parents which were either **F Hh** selfed or **F hh** × **Hh**, and it is likely therefore, because they do not occur in **HH** families, that these female shoots on hermaphrodite plants are due to mutations of **H** → **h**.

Proof of this explanation was obtained by selfing the hermaphrodite part of a plant which was **FFHh**; it gave 188 ☿ and 57 ♀s. A female shoot on the same plant was crossed by the hermaphrodite part, giving 168 ☿ and 180 ♀s. Thus showing that the female mutant part was **FFhh**.

In the later generations there occurred small numbers of hermaphrodites with all the corollas no larger than those of the females. Whether these were **ffhh** types which had lost their lethality due to selection and inbreeding or were due to rare crossing over or mutation at either the **F** or **H** loci we cannot say.

CONCLUSIONS

Two genes control sex determination in *Origanum vulgare*. The dominant gene **F** causes anther abortion and **H** is a dominant suppressor of **F**. The numerical disadvantage which this mechanism imposes on the females is counteracted in wild populations in three ways.

1. The bottom recessive **ffhh** is most probably lethal.
2. About 75% of the **HH** hermaphrodite genotypes are inviable.
3. The seed output of hermaphrodite plants is lower than that of females.
4. Mutation of the gene **H** to **h**.

In cultivation the controlled breeding and reduced competition weaken these compensating factors and consequently the proportion of hermaphrodites increases.

DISCUSSION

Possible Tetrasomic Explanations

As pointed out earlier a chromosome number of 32 could indicate tetrasomic inheritance, and two possible tetrasomic schemes have been considered. In the first, a single gene **H** is necessary for anther production. The dominance of **H** is assumed to be incomplete so that both **hhhh** and **Hhhh** would be females, and the duplex, triplex and quadriplex all hermaphrodites. The segregations expected on this system, however, are at variance with the data in two respects. Firstly, the original crosses would then have to be either **Hhhh** × **HHHh** or **hhhh** × **HHHh**, for the **HHHh** is the only true breeding hermaphrodite which will give segregating progeny when crossed onto a female. The expected ratios would be 3:1 or 1:1 respectively,

neither of which was found. Secondly, and more important, is the fact that selfing an **HHHh** plant should give hermaphrodite progeny, a quarter of which would not be true breeding. Fifteen hermaphrodites from such families were tested but all of them bred true, which is a significant deviation from expectation. For these reasons this system is ruled out.

The second scheme with tetrasomy involves the two loci F and H, the functions of which have already been defined. This type of gene action would give results with both disomy and tetrasomy which would explain the data. The ratios expected with the disomic and tetrasomic inheritance with the families obtained are not strikingly different, and it is impossible with the data to discriminate between them. For simplicity the disomic interpretation has been adopted.

Breeding Systems in Equilibrium

Calculations have been made of the equilibrium between females and hermaphrodites in a gynodioecious species, in which the control is by (1) cytoplasm, (2) a dominant gene and (3) a recessive gene (Lewis, 1941). With cytoplasmic control an equilibrium can be obtained without any additional assumptions about differential fertility. But with genic control, whether dominant or recessive, the equilibrium formula obtained was:

$$p = \tfrac{1}{2} - \frac{1}{f(1-p)^{x-1}}$$

where p is the proportion of females, $f(1-p)^x$ is the fitness of the female relative to that of the hermaphrodite, and f is the fertility. Thus if $f = 2$ then $p = 0$. Therefore the females must be more than twice as fertile as the hermaphrodite if they are to exist in the population. This calculation is based on the assumption that fertilization between gametes is at random.

The genic control of gynodioecy found in *Origanum vulgare* is neither by a single recessive nor by a single dominant gene. It is controlled by two genes, the dominant **F** which causes anther abortion and the dominant **H** which suppresses the action of **F**. To obtain an equilibrium this two-gene system would require even more support from ancillary lethality and differential fertilization than is required by either the straightforward dominant or recessive gynodioecy. The female in *Origanum* must be the recessive **hh**, which makes it similar to the gynodioecy controlled by a single recessive gene, and therefore subject to the equilibrium formula above. But it must also have the dominant gene **F**. This places an added handicap on the female, because many of the genotypes which pollinate the female have the **ff** genotype. Thus, without differential fertility or fertilization the *Origanum* system would appear to have even more difficulty in maintaining a balance between the two groups than the seemingly improbable dominant and recessive gynodioecy.

The two-gene control, giving its apparent further handicap to the system as a workable outbreeding mechanism, is the clue to the solution of the problem. Let us consider how an equilibrium can be obtained either by differential fertility or fertilization. This can best be done by examining the properties of some well known and hypothetical breeding systems.

Dioecy, found commonly in animals and less frequently in plants, and heterostyly, which is common in some plant families, both have two mating groups, one heterozygous and the other homozygous for the control genes. Each group contributes to the succeeding generation equal numbers of genes not linked with the "control" genes. But each contributes unequal numbers of "control" genes, these are in the ratio of 1:3, for example 1y to 3x chromosomes for dioecy or 1**S** to 3**s** genes for heterostyly.

The gametophytic incompatibility system as found in *Nicotiana*, etc., has a large number of different mating groups, each being a different heterozygote, $\mathbf{S}_{1.2}$,

TABLE 7. *A comparison of independent genes and control genes contributed to succeeding generations and the equilibrium expressed as ratios. Note that the product (control genes × equilibrium) may be either 1:1 or 1:3. For further details see text*

System	Genetics		Independent genes	Control genes	Equi-librium	Product (Cont. × Equil.)
Dioecy	♀ xx	♂ xy	1:1	1:3	1:1	1:3
Heterostyly	Pin ss	Thrum Ss	1:1	1:3	1:1	1:3
Gametophytic Incompatibility	$S_{1.2}$	$S_{3.4}$ etc.	1:1	1:1	1:1	1:1
Dominant Gynodioecy	♀ Ff	♀ ff	3:1	3:1	1:3	1:1
Recessive Gynodioecy	ff	Ff or FF	3:1	1:1	1:3	1:3
Incompatibility (Hypothetical)	$S_{1.2}$	$S_{2.2}$	3:1	1:2	2:1	1:1
Incompatibility Embryo-Sac (Hypothetical)	$S_{1.2}$	$S_{2.2}$	3:1	1:3	1:1	1:3

etc. Each of these groups contributes equal numbers of genes independent of the **S** gene to the succeeding generations as in dioecy and heterostyly, but the system differs in that each group also contributes on the average an equality of control genes. The equilibrium is again equality of the groups.

The relevant features of the systems are collected in table 7, from which it will be seen that the product of the two ratios, control genes and equilibrium is 1:3 in dioecy and heterostyly and 1:1 in gametophytic incompatibility. Thus from the well-known systems there are two ways of attaining equilibria, the 1:3 and 1:1.

Table 7 also includes dominant gynodioecy in which the female is **Ff** and the hermaphrodite is **ff** and the recessive gynodioecy with **ff** females and **Ff** and **FF** hermaphrodites. These two systems differ from dioecy and gametophytic incompatibility in their 3:1 ratio of genes independent of the control genes contributed to the next generation. The equilibrium of 25% of females, i.e. 1:3, has been obtained from the formula using the required relative fertility of the female to the hermaphrodite, $f(1-p)=3$. It is interesting that the two product ratios 1:3 and 1:1 found in dioecy and gametophytic incompatibility are also found not only in the two gynodioecious systems but in each of the two hypothetical incompatibility systems given in the table. Each consists of two groups, a heterozygote and homozygote, and both can maintain a balance between the groups without difficulty.

The first of these hypothetical systems is based upon a reaction between pollen and style with one gene and two alleles, $\mathbf{S}_1$ and $\mathbf{S}_2$. The pollen is under gametophytic control but in the style $\mathbf{S}_1$ is dominant over $\mathbf{S}_2$. An $\mathbf{S}_{1\ 2}$ plant is self-fertile by virtue of the $\mathbf{S}_2$ pollen not being inhibited in a $\mathbf{S}_{1\ 2}$ style where $\mathbf{S}_1$ is completely dominant over $\mathbf{S}_2$. The other group $\mathbf{S}_{2\ 2}$ is self-sterile owing to the oppositional effect of $\mathbf{S}_{2\ 2}$ style on $\mathbf{S}_2$ pollen. The group $\mathbf{S}_{1\ 1}$ is not formed because $\mathbf{S}_1$ pollen is inhibited in a $\mathbf{S}_{1\ 2}$ style.

Bateman (1952) has pointed out that in this system if the $\mathbf{S}_{1\ 2}$ plants were largely self-pollinating there would be two classes of matings:

$$\begin{array}{l} \mathbf{S}_{1\ 2}\ \text{selfed} \rightarrow \mathbf{S}_{1\ 2} \quad \mathbf{S}_{2\ 2} \\ \qquad\qquad\qquad\quad 1:1 \\ \mathbf{S}_{2\ 2} \times \mathbf{S}_{1\ 2} \rightarrow \mathbf{S}_{1\ 2} \end{array},$$

The equilibrium would be 2 $\mathbf{S}_{1.2}$:1 $\mathbf{S}_{2.2}$. The contribution of control genes to succeeding generations is 1:2, making a product of 1:1 as in gametophytic incompatibility and dominant gynodioecy. It should be noticed that the pollen of

$S_{2.2}$ is not able to participate in fertilization, and consequently there is every possibility of this genotype becoming male-sterile by the accumulation of genes causing anther abortion. This incompatibility would then become a gynodioecious system, attaining its equilibrium by the selective elimination of male gametes: for, of the pollen from an $S_{1.2}$ plant, S_2 functions twice as frequently as S_1. Because the $S_{1.2}$ group supplies not only its own female and male nuclei but also the male nucleus for the female there is a 3:1 ratio of genes independent of **S** contributed to the next generation, as in dominant and recessive gynodioecy. This incompatibility system attains its equilibrium despite this unequal contribution, by the elimination of male gametes.

The second theoretical incompatibility system is one based upon a reaction between the male and female generative nuclei in the embryo-sac similar to that found in *Theobroma cocao* (Knight and Rogers, 1955).

The pollen is under sporophytic control with S_1 dominant, the embryo-sacs are gametophytically controlled. Pollen and embryo-sacs with the same reaction are incompatible; thus an $S_{1\ 2}$ plant is self-compatible because all the pollen behaves as S_1 due to dominance, whereas half the embryo-sacs have the S_2 reaction. $S_{2\ 2}$ plants are self-incompatible because all pollen and embryo-sacs have the same reaction.

Thus,

$$S_{1\ 2} \times S_{1\ 2} \rightarrow \underset{1:1}{S_{1\ 2} + S_{2\ 2}}$$

$$S_{2\ 2} \times S_{1\ 2} \rightarrow \underset{1:1}{S_{1\ 2} + S_{2\ 2}}$$

In this system the equilibrium is 1:1. The contribution of control genes is 1:3, which gives a product (control × equilibrium) of 1:3. This is the same as in dioecy and recessive gynodioecy. The equilibrium is obtained by the fertility of the heterozygotes, $S_{1.2}$ being reduced to a half by the incompatibility of S_1 embryo-sacs. These embryo-sacs are not fertilized and sooner or later a lethal gene linked with S_1 operating in the embryo-sac would arrive and be selected, thus ensuring that all viable embryo-sacs were S_2. At this stage the equilibrium between the two groups is obtained by differential elimination of female gametes. In a similar way many ring forming *Oenothera* species with a balanced lethal system have evolved.

In both of these hypothetical but workable incompatibility systems the pollen of one group never functions, and would become sterile by the selection of a male sterility gene. Such a gene could either be a recessive linked with S_2, as postulated by Bateman (loc. cit.), or could be an independent dominant gene but hypostatic to S_1 as found in *Origanum*. They are therefore systems which could be the starting point for the evolution of gynodioecy.

If we compare the control of gynodioecy in *Origanum* with these incompatibility systems it will be seen that the correspondence with the first system based upon style and pollen reactions is not complete, but with the second based upon the embryo-sac the fit is nearly perfect.

With the first system $S_{1\ 2}$ would be the hermaphrodite which is **Hh** in *Origanum*, $S_{2\ 2}$ would be the female which is **hh**. The selfed hermaphrodites should give one hermaphrodite to one female, and the females should give all hermaphrodites. Since true breeding hermaphrodites and segregating females are the rule in *Origanum* this system does not apply.

The second incompatibility system based on the embryo-sac should give segregating females and both segregating and non-segregating hermaphrodites, which is in complete agreement with the results in *Origanum*. On the assumption that the two gene *Origanum* system has evolved from the embryo-sac type of incompatibility then **H** is analogous to S_1 and **h** to S_2. The rarity of **HH** types found in *Origanum* is in complete agree-

ment with expectation because S_1 pollen should not normally fertilize S_1 embryo-sacs. Furthermore the self-incompatibility of the **HH** genotype was a striking fulfilment of the prediction deduced from the incompatibility system. Such a rare **HH** type would be the result of an occasional illegitimate fertilization, which is found in most incompatibility systems of an **H** embryo-sac. The viability of this embryo-sac could be the result of a cross-over between the **H** locus and the postulated embryo-sac lethal.

Because gynodioecy could have evolved gradually from incompatibility through several stages which are all workable breeding systems, the main difficulty inherent in a breeding system with groups which contribute such unequal amounts of genetic material is removed.

Correns (1908) found a remarkable situation in *Plantago lanceolata*, the females gave 1:1 of females and hermaphrodites and the hermaphrodites bred true and were self-sterile. Clearly there is here also a connection between incompatibility and gynodioecy.

East (1934) put forward an alternative to the usual cytoplasmic explanation for the gynodioecy in *Satureia* and *Circium* by postulating genic control, selective fertilization and elimination of gametes. This does not differ in principle from the explanation for *Origanum*. For example he postulated that the hermaphrodite was **aa** and bred true the female was **Aa** and that **a** gametes of the female were either not viable or were not fertilized. In view of the findings in *Origanum* this seemingly unlikely explanation of East is not so unreasonable and deserves reinvestigation.

Summary

1. Gynodioecy — interbreeding hermaphrodites and females—is an outbreeding mechanism which differs from other systems in that the two mating types contribute unequal numbers of genes to the next generation.

2. *Origanum vulgare* has a gydodioecious system which is controlled by the segregation of two independent genes. The dominant gene **F** causes anther abortion and the gene **H** is a dominant suppressor of **F**. Females are therefore **Ffhh** or **FFhh**, hermaphrodites are **FfHh**, **FfHH**, **ffHh** or **FFHH**.

3. The bottom recessive **ffhh** has not been found and is presumed to be lethal. The **HH** genotype arises in the proportion of one-quarter of the expected, and this genotype is self-incompatible.

4. The lethality and reduced production of these genotypes together with a lower seed output of the hermaphrodite are factors of importance in the maintenance of the two types in nature.

5. The genetic control in all its details supports a hypothesis that gynodioecy in *Origanum* has evolved from an incompatibility system with sporophytic pollen control and inhibition of fertilization in the embryo-sac.

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THE ORIGIN OF CLARKIA LINGULATA

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An unusual *Clarkia* was discovered in 1947 in the canyon of the Merced River, California, by the senior author. It appeared to be a simple variant, with unlobed petals, of *C. biloba* (Dur.) Nels. & Macbr., a species which occurs abundantly in the same canyon. Examination of microsporocytes showed, however, that this variant had genomes of 9 chromosomes, rather than the 8 characteristic of *C. biloba*. Furthermore, the hybrid between them was nearly sterile. Consequently, the new entity was described as a species, *C. lingulata* (Lewis and Lewis, 1953).

The purpose of this paper is to compare these two species phenotypically and cytogenetically in order to ascertain the factors which led to speciation.

DISTRIBUTION AND BREEDING HABIT

Clarkia lingulata is known only from two sites in the Merced River Canyon. One population is on the east and north-facing slopes at the junction of the Merced River with its South Fork; the other population is on a steep north-facing slope about two miles farther down the canyon. Other populations may occur, but the deep and steep-sided canyons of the Sierra Nevada are difficult to explore, except where roads or trails exist. On the other hand, *C. lingulata* may be endemic to a very small area and is perhaps confined to the two known colonies. Each of these colonies is morphologically homogeneous and consists of several thousand flowering individuals in an ordinary season.

Clarkia biloba, on the other hand, is a polytypic species with an area of distribution which extends for about 150 miles along the Sierra Nevada foothills from Butte County to the Merced River. It is frequent throughout this area and occurs in more or less discrete colonies of various size. A few colonies are also found in a disjunct area near San Francisco Bay (fig. 1).

The southern limit of distribution of *C. biloba* coincides with the restricted distribution of *C. lingulata* and colonies of the two species occur within 100 yards of each other, although no mixed colonies are known. This absence of mixed colonies, despite the close proximity of the two species, suggests a difference in ecological preference. However, the sites occupied by adjacent colonies of the two species appear to be comparable in all respects and a closely related species, *C. dudleyana*, grows with both species in this area. Furthermore, under uniform cultural conditions no differences in response between *C. biloba* and *C. lingulata* have been detected. Nevertheless, effective ecological differences between the species are not precluded, even though our observations show no gross differences.

Both species are insect pollinated. The anthers mature before the stigma is receptive and the stigma is held well above the anthers. Consequently, out-crossing is the rule, but individuals of both species are self-compatible and self-pollination can occur from insect visits to more than one flower on the same plant. The seeds have no special mechanism for dissemination and are generally dropped close to the parent plant.

MORPHOLOGICAL STUDIES

Clarkia biloba consists of three geographical races designated as subspecies *brandegeae*, *biloba* and *australis* (fig. 1),

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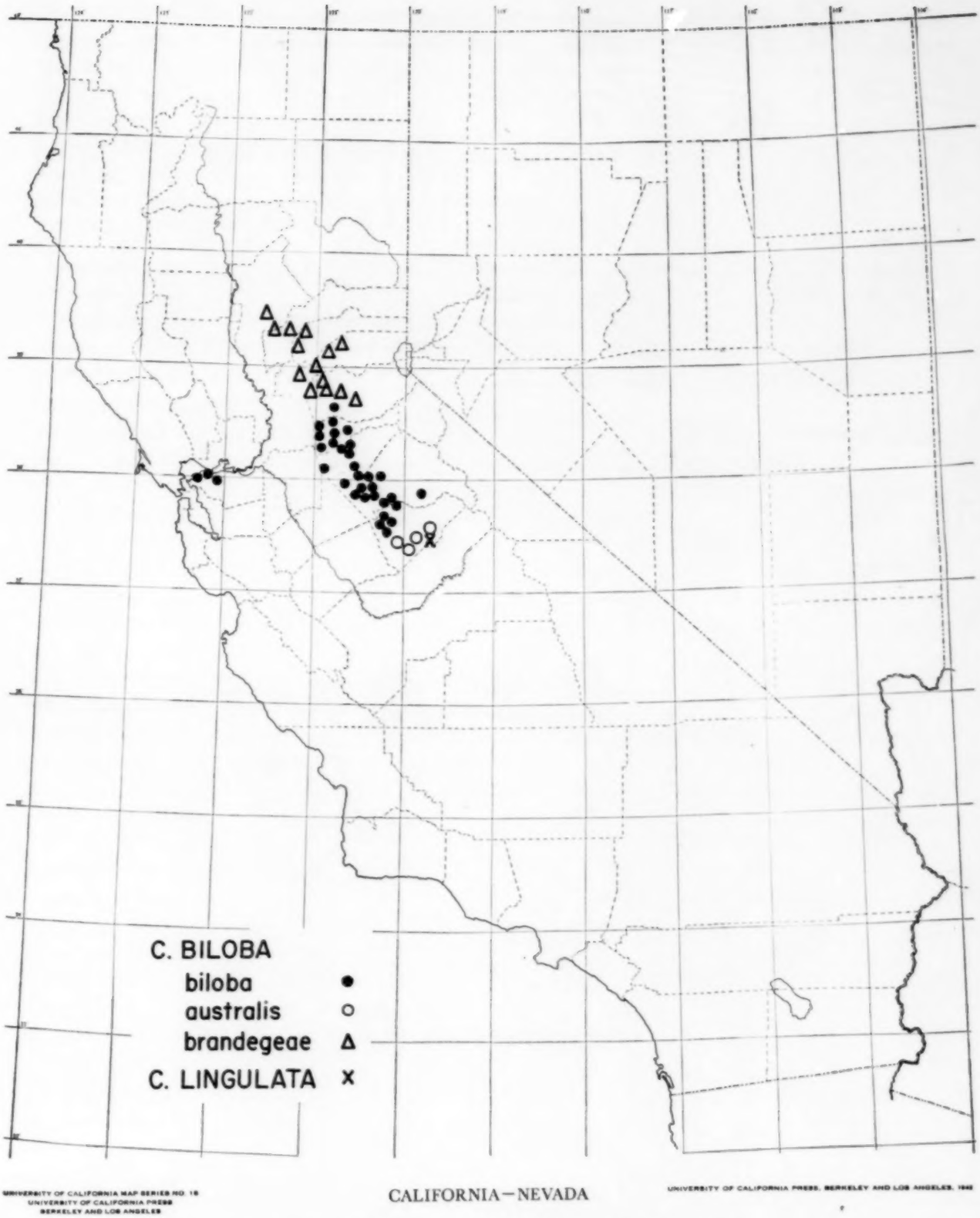

FIG. 1. Distribution of *Clarkia biloba* and *C. lingulata*

which we shall refer to without specific epithet. These subspecies differ from one another most conspicuously in petal shape and color, although vegetative differences are also discernible (Roberts and Lewis, 1955). Measurements of the more conspicuous morphological traits are summarized in table 1. From this table it will

TABLE 1. *Morphological characters of* Clarkia lingulata *and* C. biloba

Species	Petal length / breadth	Petal sinus depth / petal length	Petal color	Sepal length (mm.)	Leaf breadth (mm.)	Leaf margin	Stem tip
C. lingulata	1.9 (2.51) 3.1*	.00 (.00) .08	magenta	7 (9.0) 10	3 (4.9) 8	subentire	recurved
C. biloba							
australis	1.4 (2.23) 3.3	.18 (.23) .31	magenta	7 (9.1) 11	3 (4.4) 6	subentire	recurved
biloba	1.2 (1.71) 2.4	.22 (.34) .52	pink to lavender	6 (11.5) 17	5 (5.9) 8	subentire	recurved
brandegeae	1.2 (1.69) 2.3	.09 (.15) .22	lavender to orchid	9 (12.4) 15	5 (7.1) 8	denticulate	strongly recurved

* Minimum, mean, and maximum are indicated for ratios and measurements.

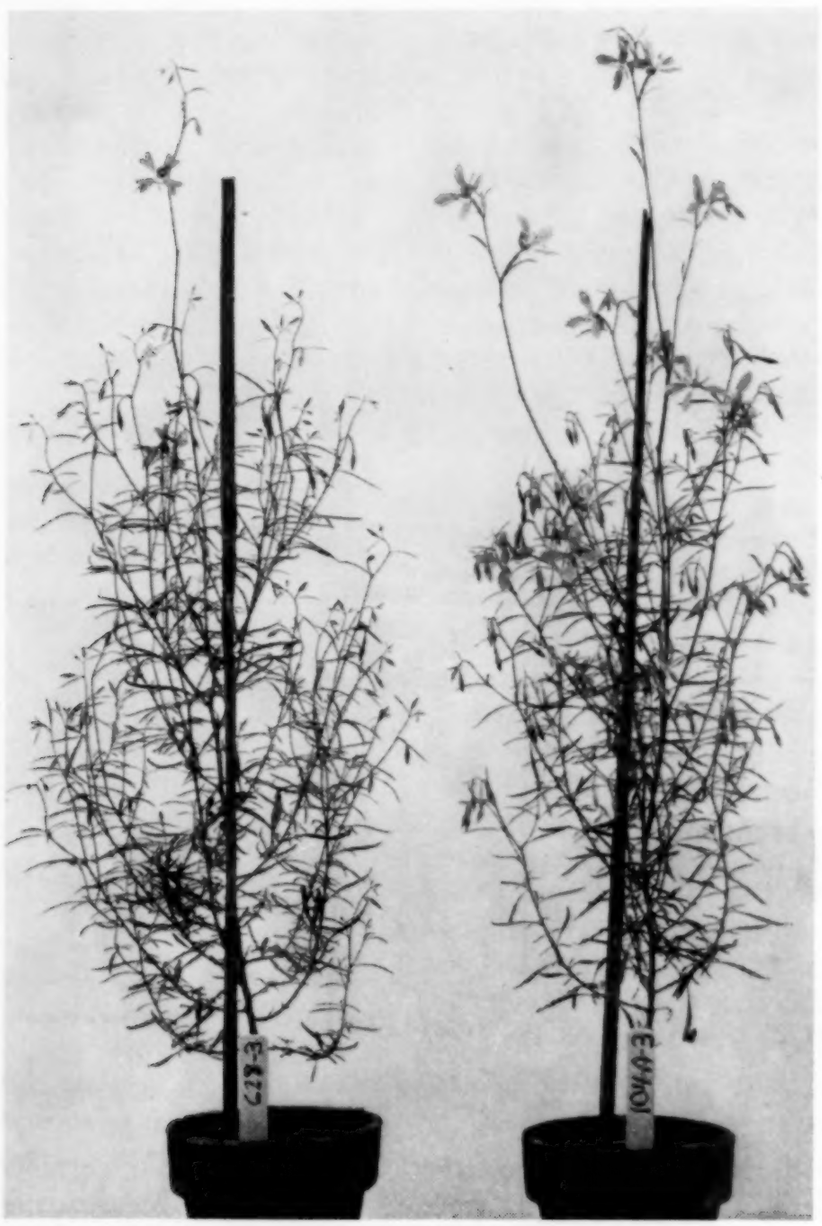

FIG. 2. *Clarkia biloba australis* (left) and *C. lingulata* (right) grown simultaneously under the same cultural conditions.

be seen that the range of variation of *C. lingulata* with respect to vegetative characters as well as petal color and proportion falls within that of *australis*, the southernmost subspecies. In fact, *C. lingulata* differs from this subspecies in external morphology only by the degree to which the petals are lobed, as shown in figure 2.

Chromosome Studies

Meiosis has been studied in squash preparations of microsporocytes of thirty-seven individuals of *C. lingulata* and one hundred fourteen individuals of *C. biloba*, including all of the subspecies (Roberts and Lewis, 1955). All of the individuals of *C. lingulata* were found to have 9 pairs of chromosomes at first metaphase, whereas the individuals of *C. biloba* consistently had 8. Seven of the nine pairs in *C. lingulata* usually form ring bivalents, with chiasmata in both arms; the other two pairs usually do not. In *C. biloba* only six of the eight pairs usually form ring bivalents. This suggests that the genomes of the two species differ by a chromosome that normally forms chiasmata in both arms and, hence, has a submedian centromere. This conclusion is further substantiated by the chromosome configurations found in the F_1 hybrids discussed below.

Mitotic chromosomes of the two species have been studied in root-tip sections. Individual chromosomes could not be distinguished but careful measurement of the entire metaphase complement indicates that the somatic complement of *C. lingulata* differs from that of *C. biloba* by two chromosomes of average length (Lewis, 1954).

Interspecific Hybridization

A total of sixty crosses have been made between *C. lingulata* and the three subspecies of *C. biloba*. The results are shown in table 2. The seed set resulting from crosses between *C. lingulata* and *australis* was as high as the norm for the parents, but was lower in crosses with the other two subspecies, particularly *brandegeae*. All of the seeds from these original crosses and subsequent hybrid progenies germinated equally well.

The F_1 hybrids are morphologically intermediate in all respects. The fertility of these hybrids is low, as indicated by the percentage of visibly good pollen and by seed set. The percentage of good pollen in the parental plants was in all instances above 90, and usually above 95 per cent. In contrast, good pollen did not exceed 15 per cent in hybrids between *C. lingulata* and *australis*. Similar results were obtained for hybrids with *biloba*. On the other hand, the hybrids with *brandegeae* consistently showed a higher percentage of good pollen (table 2). Comparable results were obtained with respect to seed set. The hybrids with *australis*, when self-pollinated, set only one capsule containing one seed, despite 33 pollinations; the hybrids with *biloba* did but little better. In contrast, the hybrids with *brandegeae* produced as many as 9

Table 2. *Fertility of the* F_1 *hybrids between* Clarkia lingulata *and the three subspecies of* C. biloba

C. lingulata × *C. biloba*	*australis*			*biloba*			*brandegeae*		
	n	M	$\bar{X}$	n	M	$\bar{X}$	n	M	$\bar{X}$
seed set—original cross	39	73	39.6	3	30	22.0	8	25	18.6
per cent good pollen—F_1	23	15	9.4	3	14	12.0	18	27	16.3
seed set—F_1 × self	33	1	<1	18	3	<1	12	9	5.0
seed set—F_1 × parent	55	19	2.2	29	13	1.5	30	32	9.5

seed set: n = number of pollinations; M = maximum number of seeds per capsule; $\bar{X}$ = mean number of seeds per capsule.

per cent good pollen: n = number of plants; M = maximum; $\bar{X}$ = mean per plant.

seeds per capsule and nearly every pollination resulted in a capsule. When backcrossed, the hybrids with *brandegeae* again proved to be conspicuously more fertile than the hybrids with the other two subspecies. An explanation of these results is to be found, at least in part, from a study of meiosis in the hybrids.

Meiosis in the hybrids between *C. lingulata* and each of the three subspecies of *C. biloba* will be discussed in turn. In all instances squash preparations of microsporocytes were used. In contrast to the parents, the meiotic chromosomes of the hybrids and subsequent progenies tend to be "sticky" which frequently makes an analysis of configurations difficult.

Five hybrids between *C. lingulata* and *australis* have been examined. The parents were obtained from two different colonies of each. Since meiosis was found to be comparable in each hybrid, the data obtained have been combined. The configurations at first metaphase could be examined with certainty in 94 microsporocytes. The maximum association was found to be 4 pairs, a ring of 4, and a chain of 5 chromosomes (fig. 3 d, e).

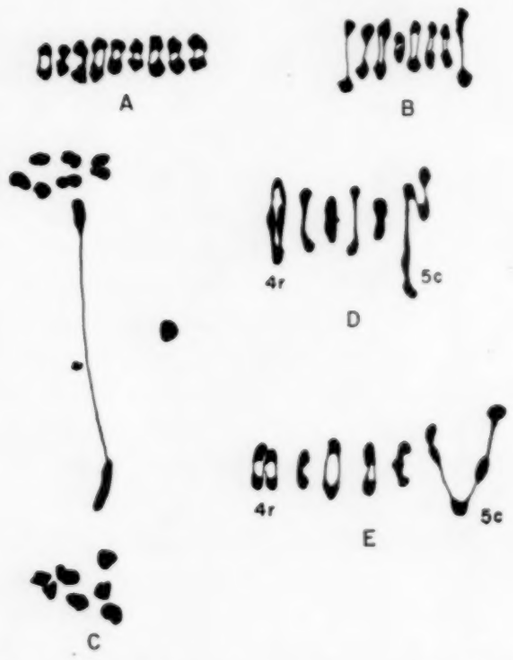

FIG. 3. Meiotic chromosomes of *Clarkia lingulata, C. biloba australis,* and their F_1 hybrid.

A. *C. lingulata,* metaphase I; B. *C. biloba australis,* metaphase I; C. F_1 hybrid, anaphase I, showing a bridge, fragment, and lagging chromosome; D. and E. F_1 hybrid, metaphase I, showing four pairs, a ring of 4, and a chain of 5.

Four pairs of chromosomes were present in every cell and always separated in a regular manner. This suggests that each of these pairs may be comprised of structurally homologous chromosomes. If so, these four pairs do not contribute to the observed reduction in fertility of the hybrid.

The ring of 4 chromosomes indicates that the parental plants differ by a reciprocal translocation involving large parts, if not entire arms, of two pairs of chromosomes. In addition to a ring of 4, these chromosomes form a chain of 4 or two pairs. The observed frequency of these configurations was 74, 16, and 10 per cent respectively. In no instance was a chain of 3 and an unpaired chromosome observed. When a ring of chromosomes is formed it may separate disjunctionally at anaphase, with adjacent chromosomes moving to opposite poles (fig. 3e), or it may separate nondisjunctionally, with adjacent chromosomes moving to the same pole (fig. 3d). In nearly all cases of nondisjunction, the ring was observed to be oriented in such a manner that two chromosomes on opposite sides of the ring were toward the poles, with the other two chromosomes more or less on the equator. Sixty five per cent of the rings were observed to separate nondisjunctionally (table 3), and these would not be expected to produce viable genotypes. When a chain rather than a ring was formed, it was observed to separate nondisjunctionally in about half of the cells. When two separate pairs are formed, they are presumably oriented independently, and half of the time would not be expected to produce viable genotypes. In total, then, one would expect approximately 62 per cent of the meiotic products of the cells observed to be nonfunctional because of the translocation alone. However, the cells that could be analysed cytologically may not represent a random

TABLE 3. *Chromosome configurations in microsporocytes of* F_1 *hybrids between* Clarkia biloba *and* C. lingulata

T	∩∪	∞	∩∪	|	∩|	∪∪	||	Total number of cells
aust. x ling.	45	25	7	6	2	0	9	94
brand. x ling.	50	31	6	1	0	1	9	98

L	∨∪|	∪∪|	∨∪∪	∩|	∪∪∪	∩∪∪	∩∪∩	∨∨|	|||	|||	||||	Total number of cells
aust. x ling.	21	18	6	2	2	0	1	13	25	0	6	94
brand. x ling.	1	1	2	0	1	1	1	65	15	1	10	98

T = translocation configurations. L = configurations involving the "ninth" chromosome of *C. lingulata*. aust. = *C. biloba australis*. brand. = *C. biloba brandegeae*. ling. = *C. lingulata*.

sample. Consequently, the fertility of backcross hybrids, discussed below, that are presumably structurally homozygous except for this translocation probably affords a better measure of its effect upon fertility.

The 5 chromosomes that form a maximum configuration of a chain of 5 obviously include the additional, ninth, chromosome of *C. lingulata*. This maximum configuration was observed in 53 per cent of the cells. These same 5 chromosomes were observed to form a chain of three and a pair, or two pairs and an unpaired chromosome in 41 and 6 per cent of the cells respectively (table 3). In one cell a chain of 4 and an unpaired chromosome was found, but the position of the unpaired chromosome suggested that it may have separated precociously from the chain of 4.

The various metaphase associations of these 5 chromosomes are most easily explained by the assumption that the additional chromosome of the *C. lingulata* genome is homologous, at least so far as the pairing segments are concerned, with parts of two chromosomes that are common to the genomes of both *C. lingulata* and *australis* (fig. 4). With this interpretation, one would expect the completely homologous chromosomes to be regularly associated at meiotic first metaphase in the hybrid; the additional chromosome from *C. lingulata* would be unpaired, or more frequently, would be associated with one or the other pairs, with which it is partly homologous, to form a chain of 3; or it would join the two pairs together to form a chain of 5.

The chain of 5 chromosomes is oriented in various ways (table 3). Some orientations would be expected to dissociate in such a manner as to result in viable products, whereas others certainly would not. Since we do not know the frequency with which nondisjunction and elimination of chromosomes may occur for some of these orientations we cannot estimate with any degree of accuracy the reduction in fertility that would be expected. However, the data obtained from backcross progeny, discussed below, indicate that the reduction in fertility from the dissociation of these five chromosomes is appreciable and probably sufficient, when combined with the effects of the translo-

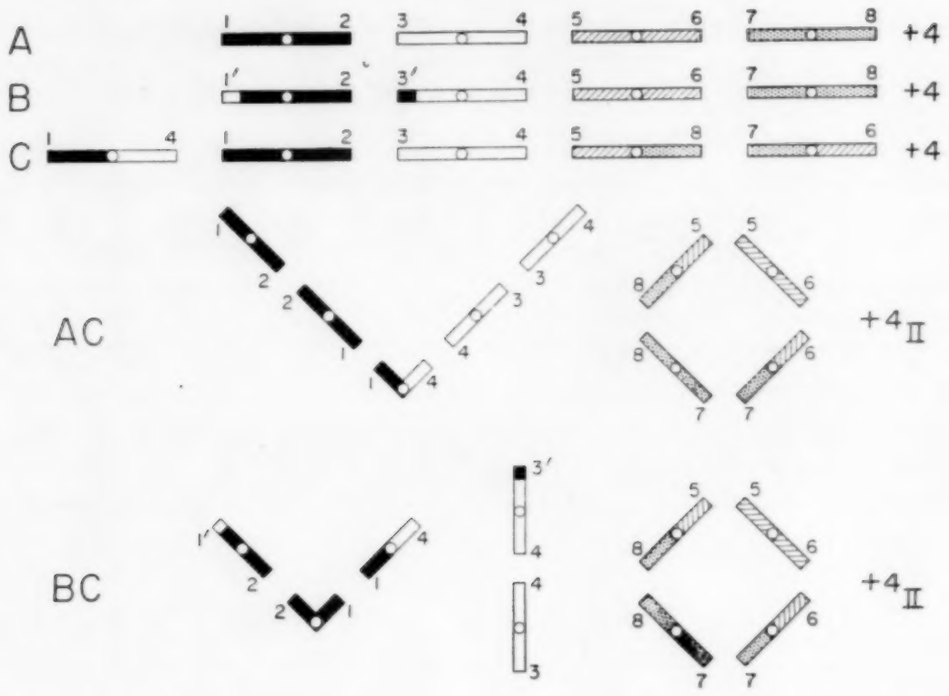

FIG. 4. Diagram of the structural and quantitative relationship of the genomes of (A) *Clarkia biloba australis* and *C. biloba biloba,* (B) *C. biloba brandegeae,* and (C) *C. lingulata.*

An inversion, not shown, differentiates genomes A and B from C but the chromosome in which it is located is not known.

cation, to account for the observed reduction in fertility of the hybrid.

A bridge and an accompanying fragment was observed at first anaphase in one hybrid cell (fig. 3c), indicating that the genomes concerned differ by a paracentric inversion. Since the same configuration is also occasionally found in cells from other hybrid progenies it seems probable that the two species differ consistently by at least one paracentric inversion. However, because of the low frequency with which crossing over apparently occurs within the inversion, its effect upon fertility is probably negligible.

Meiosis in hybrids between *C. lingulata* and *biloba* was found to be comparable in all respects to meiosis in the hybrids between *C. lingulata* and *australis.* The same configurations appear in both and in comparable frequencies.

It has been shown elsewhere (Roberts and Lewis, 1955) that the two subspecies, *biloba* and *australis,* are completely interfertile and that hybrids between them regularly form 8 bivalents. Consequently, the earlier conclusion that these two subspecies have structurally comparable genomes is further substantiated by the similarity of behavior of their genomes and comparable reductions in fertility in hybrids with *C. lingulata.*

Meiosis has been studied in two hybrids between *C. lingulata* and *brandegeae.* A total of 98 cells have been analysed. The maximum chromosome association was found to be 4 pairs, a ring of 4, and a chain of 5 chromosomes, exactly as in the hybrids discussed above. The ring of 4 was found in approximately the same percentage of microsporocytes and showed a comparable frequency of nondisjunctional separations (table 3). The conclusion seems justified that the ring has the same structural basis in all of the hybrids and that its effect in reducing fertility is essentially the same in each. On the other hand, a chain of 5 chromosomes was found infrequently (table 3). In most of the microsporocytes these 5 chromosomes formed a chain of 3 and a pair, and, in the remainder, two pairs and an

unpaired chromosome. One explanation of the low frequency with which a chain of 5 is formed would be that a structural rearrangement is involved which greatly restricts chiasma formation between two particular chromosome arms.

We have shown elsewhere (Roberts and Lewis, 1955) that hybrids between *brandegeae* and each of the other two subspecies of *C. biloba* are approximately 50 per cent sterile, although eight pairs of chromosomes are regularly formed at meiosis. We have suggested, as a possible explanation, that they may differ by a translocation of a sort that, in heterozygotes, greatly restricts chiasma formation in the chromosome arms involved. The result would be that two pairs rather than a ring of 4 chromosomes would usually be formed and the independent orientation of these pairs would lead to deficiencies in half of the products. Should this presumed translocation include the same chromosomes that comprise the additional chromosome in the genome of *C. lingulata,* a chain of 3 and a pair, rather than a chain of 5, would be the normal maximum association in the hybrid with *brandegeae.*

The structural relationship of the genomes of *C. lingulata* and the three subspecies of *C. biloba,* suggested by their behavior in hybrid combinations, is diagramed in figure 4. The evidence indicates that the genome of *C. lingulata* is structurally more similar, and hence more closely related to that of subspecies *australis* and *biloba* than it is to *brandegeae.* Nevertheless, as we have shown above, hybrids with the latter are the most fertile. The explanation of this anomalous situation probably lies in the frequency with which chains of 5 chromosomes are formed in the different hybrids. Additional evidence that this is indeed the case

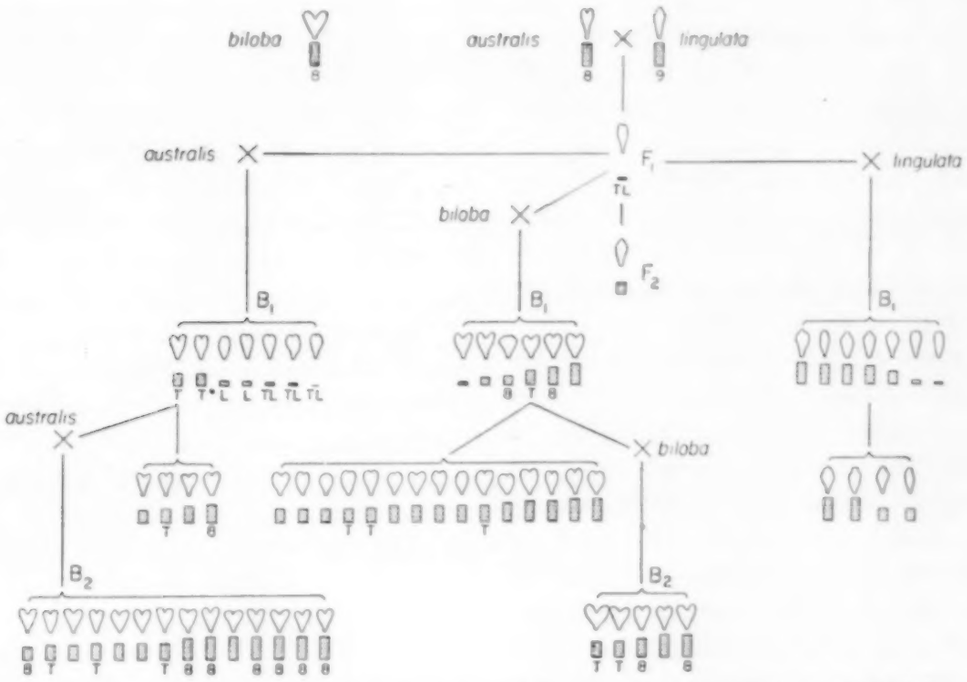

FIG. 5. Tracings of representative petals of *Clarkia lingulata, C. biloba australis,* their F_1 hybrid, *C. biloba biloba,* and the indicated progenies.

Progenies derived from self-pollination are shown by a direct line from the parent. Fertility is indicated by bar diagrams. Known chromosome complements are indicated by the following symbols: 8—structural homozygotes with 8 pairs of chromosomes; 9—structural homozygotes with 9 pairs of chromosomes; T—heterozygous for a reciprocal translocation; L—heterozygous for the "ninth" chromosome of *C. lingulata;* *—17 chromosomes but not heterozygous for the "ninth" chromosome of *C. lingulata.*

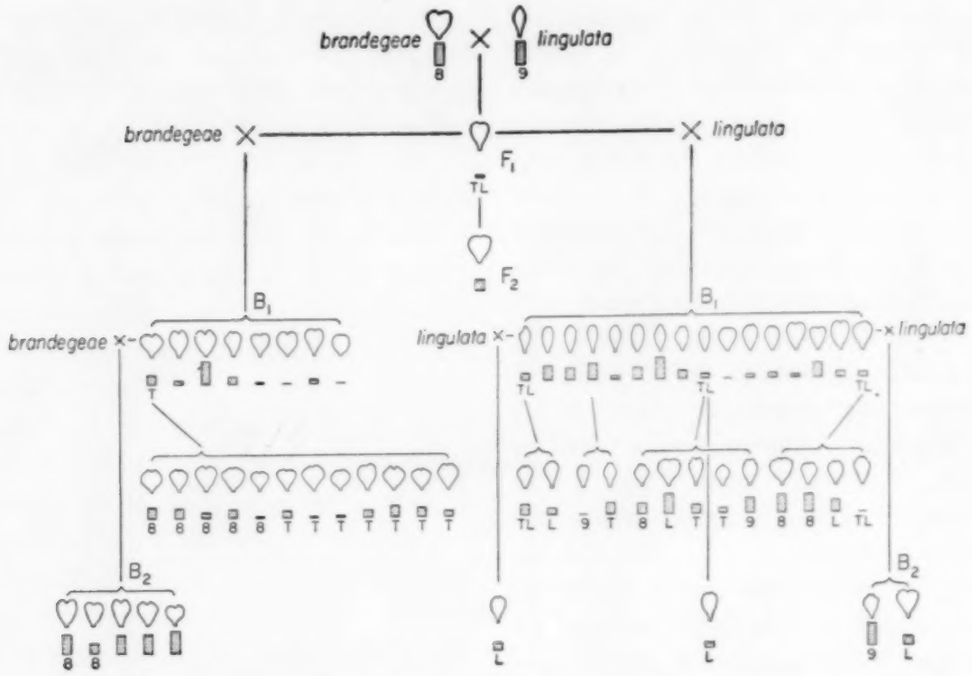

FIG. 6. Tracings of representative petals of *Clarkia lingulata, C. biloba brandegeae,* their F_1 hybrid, and the indicated progenies.

Progenies derived from self-pollination are shown by a direct line from the parent. Fertility is indicated by bar diagrams. Known chromosome complements are indicated by the following symbols: 8—structural homozygotes with 8 pairs of chromosomes; 9—structural homozygotes with 9 pairs of chromosomes; T—heterozygous for a reciprocal translocation; L—heterozygous for the "ninth" chromosome of *C. lingulata.*

comes from the progenies obtained from backcrossing.

BACKCROSS PROGENIES

Progenies have been obtained from backcrossing hybrids between *C. lingulata* and each of the subspecies of *C. biloba.* Representative progenies are shown in figures 5 and 6. The petal shape of each individual is shown, together with a bar diagram indicating fertility, measured as the per cent of visibly good pollen. The chromosome constitution, if known, is also indicated. The size of the progenies shown is determined by the data available and is not indicative of relative fertilities.

Petal shape falls into two classes in the progeny obtained from backcrossing *C. lingulata* × *australis* hybrids to the *australis* parent. One group of individuals is intermediate between the two original parents; the other group is indistinguishable from *australis.* The available data indicate that the former are heterozygous for the additional chromosome from the *C. lingulata* genome whereas the latter do not have this chromosome. Furthermore, the progeny obtained from selfing a B_1 individual that did not have the additional chromosome, showed no evidence of the petal shape of *C. lingulata,* nor did the B_2 from the same individual. At the same time, these progenies did show segregation for the translocation that distinguishes the genomes of the original parents.

These data indicate that the genetic factor, or factors, determining the difference in petal shape between *C. lingulata* and *australis* is probably associated with the additional chromosome of *C. lingulata* and is certainly not associated with the translocation.

The F_1 hybrid from which the above progenies were derived was also crossed

to *biloba* (fig. 5). The resulting progeny and its derivatives were variable in petal shape but a distinction can not be made between the variation resulting from recombination of the factors responsible for the differences between the two subspecies of *C. biloba* and those attributable to *C. lingulata.*

Progenies derived from the hybrid between *C. lingulata* and *brandegeae* vary greatly in petal shape (fig. 6). Furthermore, this variation is in large measure independent of segregation not only of the translocation but also of the additional chromosome from *C. lingulata.* This indicates that the factors determining the differences in petal shape between the two original parents are several or perhaps many in number and are distributed on several and perhaps all of the chromosomes.

Segregation for fertility also follows a different pattern in the progenies from different hybrids. We have indicated above that the genomes of *australis* and *biloba* are structurally homologous and have shown that they produce hybrids of comparable fertility when crossed to *C. lingulata.* Consequently, progenies involving these two subspecies will be considered together, as regards fertility, in contrast to the progenies involving *brandegeae.*

The progenies derived from F_1 hybrids between *C. lingulata* and *australis* or *biloba* fall into a few more or less distinct classes with regard to fertility, and these show a close correspondence to the chromosome complement.

Twelve individuals of hybrid derivation shown in figure 5 regularly formed 8 pairs of chromosomes at meiosis, and these individuals were as fertile as the original parents, with two exceptions. The lower fertility in these two instances may have been due to any one of several reasons affecting the health of the plant and not to genetical or chromosomal differences. On the other hand, all of the individuals known to be heterozygous for the translocation or the additional chromosome of *C. lingulata* were conspicuously less fertile than the original parents. Twelve individuals were heterozygous for the translocation but did not have the additional chromosome from *C. lingulata.* These individuals were remarkably similar in fertility for the mean percentage of visibly good pollen, for each individual fell between 55 and 70, and most frequently near 65 per cent. These figures probably afford a much better estimate of the effect of heterozygosity for this translocation on fertility than do the figures obtained from observations of meiosis. Two individuals that had the additional chromosome from *C. lingulata* but were not heterozygous for the translocation had means of 20 and 25 per cent visibly good pollen. These figures can be taken as indicative of the effect of this additional chromosome on fertility. The validity of the estimates of the effects of the additional chromosome on fertility as well as heterozygosity for the translocation is further substantiated by the fertility of individuals that are heterozygous for both. The fertility of these individuals is comparable to that of the F_1.

The chromosome complement of one of the B_1 individuals deserves special comment. It was heterozygous for the translocation and had a total of 17 chromosomes. However, the additional chromosome was in no instance associated with a chain of 5 but at most a chain of 3 and usually was found as a univalent. In this respect, the chromosome association was comparable to that in the primary trisomics of *C. unguiculata* (Vasek, 1955) and like them had little, if any, effect on fertility. Consequently, we believe that this plant is a primary trisomic of the sort that would be expected from certain of the gametes resulting from the dissociation of the chain of 5 in the F_1 (i.e. 1-2, 3-4, 3-4, or 1-2, 1-2, 3-4).

The restoration of complete fertility in hybrid derivatives that regularly form 8 pairs of chromosomes, together with the constancy of the reduction in fertility associated with each of the two major chro-

mosomal differences indicates beyond reasonable doubt that the level of fertility of the F_1 hybrid and subsequent progenies is attributable to the two gross chromosomal differences between the genomes of the parents.

Derivatives of the F_1 hybrid between *C. lingulata* and *brandegeae* (fig. 6) are much more variable in fertility than the progenies discussed above and furthermore bear no simple relationship to the observed chromosomal differences between individuals. Heterozygosity for the translocation probably has a comparable effect upon fertility wherever it occurs. Consequently, the greater variability of these hybrids is probably due, in considerable measure, to the structural differences in the 5 chromosomes that usually form a chain of 3 and a pair rather than a chain of 5. Assuming that the structural relationships indicated in figure 4 are correct, functional gametes from the hybrid between *C. lingulata* and *australis* that include two of these chromosomes will be structurally identical (i.e., 1-2, 3-4), whereas those with three will be at most of 3 sorts (i.e., 1-2, 3-4, 1-4; 1-2, 3-4, 3-4; or 1-2, 1-2, 3-4). On the other hand, the corresponding 5 chromosomes in the hybrid between *C. lingulata* and *brandegeae* can produce two kinds of functional gametes with two of these chromosomes and seven presumably viable combinations of three chromosomes. The fertility of the individuals resulting from these nine classes of gametes will vary, depending upon the meiotic behavior and genotypes of the combinations concerned. The progenies would, consequently, be expected to be more variable in fertility and to have fewer individuals recovering full fertility, as indeed is the case. However, all of the variability in fertility in these progenies is apparently not attributable to the structural and quantitative differences we have been considering. Many of these derivatives show various degrees of asynapsis, which in the most extreme cases results in a high degree of sterility, and probably accounts for most, but not necessarily all, of the instances in which the derivatives are more sterile than the F_1 hybrid. We believe that this asynapsis results from genetic recombination rather than from structural or quantitative differences. Genetic recombination may also modify fertility in other ways in these hybrid derivatives.

Discussion

We have shown that only the shape of the petal distinguishes the external morphology of *C. lingulata* from *C. biloba australis*. Nevertheless, their respective genomes differ quantitatively by one chromosome that is homologous to parts of two chromosomes in the normal complement of both entities. The fertility of the hybrid between them is low, which we regard as a consequence of the dissociation at meiosis of the translocation ring and of the chain involving the additional chromosome. The genetical basis for the difference in petal shape is also probably associated with this additional chromosome.

The genomes of subspecies *australis* and *biloba* are apparently structurally homologous in all respects and when combined with a genome from *C. lingulata* yield hybrids of comparable fertility. However, *biloba*, in contrast to *australis*, differs from *C. lingulata* by several morphological traits, the inheritance of which is not associated with the additional chromosome of *C. lingulata*.

In addition to the differences that distinguish the genome of *C. lingulata* from those of *australis* and *biloba*, the genomes of *C. lingulata* and *brandegeae* probably differ by a translocation. This translocation involves the chromosomes from which the added chromosome of *C. lingulata* was derived. Our data also indicates that the morphological differences between *C. lingulata* and *brandegeae* are determined by genes distributed throughout the genome and that genetic recombination, as well as structural differences in the genomes, is probably responsible for a reduction in fertility of some of the hybrid

derivatives. Nevertheless, the F_1 hybrids between *C. lingulata* and *brandegeae* are more fertile than those with *australis* or *biloba;* at the same time, hybrids with *brandegeae* are more difficult to obtain.

The difficulty of obtaining the hybrid between *C. lingulata* and *brandegeae* reflects, we believe, the degree of genetic difference between the two parents. On the other hand, the greater fertility of the hybrid is due, paradoxically, to greater structural difference in the genomes which leads to more simple chromosome configurations at meiosis, and which, in turn, results in a greater number of functionable gametes in the F_1. However, the backcross progenies, in contrast to those involving *australis* or *biloba,* include a far lower percentage of individuals that recover full fertility.

Considering both morphological and cytogenetical evidence, there can be no doubt that *C. lingulata* is most closely related to the subspecies of *C. biloba* that grows adjacent to it. Let us now speculate on the way they became differentiated.

The quantitative difference between the genomes of *C. lingulata* and *australis* indicates that the former is derived from a genome of 8 by the addition of one chromosome. We know that chromosomes in excess of the basic number, particularly in *Clarkia,* do not necessarily disturb normal development (Lewis, 1954). Furthermore, other evidence also indicates that in *Clarkia* genomes of 9 have been derived from those with 8, which in turn are derivatives of an original basic number of 7 (Lewis, 1953b).

The genome of *C. lingulata* is assuredly derived from one comparable to that of *australis* for at present they apparently have 6 chromosomes in common that have not become differentiated structurally, except perhaps for an inversion, nor genetically to any apparent extent. Furthermore, the reciprocal translocation by which two pairs of chromosomes have become differentiated is not associated with any detected phenotypic difference. Consequently, the genetic differentiation between these two entities is associated primarily, if not entirely, with the additional chromosome in the genome of *C. lingulata.*

This additional chromosome may have originated as a tertiary trisomic of *australis* from which a constant tetrasomic condition was then derived. The alternative would be that the additional chromosome was derived from another, closely related, species. Two species closely related to *australis* grow with or near it, namely, *C. dudleyana* and *C. modesta.* One of these, *C. modesta,* has a petal shape suggestive of *C. lingulata.* However, we have been unable, after innumerable attempts, to obtain a hybrid between *australis* and *C. modesta* or *C. dudleyana.* In the absence of positive evidence of an interspecific origin of the additional chromosome, we are inclined to favor the first hypothesis. Furthermore, evidence from another species supports this view. A tertiary trisomic was found among the self progeny of an individual of *C. unguiculata.* The parent had the normal number of chromosomes but was heterozygous for a translocation (Lewis, 1954). However, this particular trisomic had no distinctive morphological traits.

Differentiation in petal shape between *C. lingulata* and *australis* was probably coincident with the establishment of the additional chromosome. Certainly the morphological differentiation did not preceed this establishment, although it may be due to subsequent mutation.

The translocation which differentiates the genomes of *australis* and *C. lingulata* may have become fixed before or after the difference in chromosome number but the sequence can not be determined. However, the paracentric inversion, by which these genomes differ, probably had its origin in *C. lingulata,* or a population destined to become that species, subsequent to the establishment of one or both of the chromosomal alterations which restrict genetic exchange between populations. On the other hand, the colonial

habit of these plants, of itself, may form a highly effective barrier to gene exchange, even when the distance between colonies is small (Lewis, 1953a).

The relative adaptedness of the altered genotypes that ultimately resulted in the differentiation of *C. lingulata* may have been an important factor in speciation although fixation by chance may also have played a major role. The colonial habit and the capacity for self-pollination are undoubtedly factors that would tend to promote the latter. In any event, the transition from one species to the other seems to have been a rapid process which depended only upon the fixation of one or perhaps two chromosomal alterations, which also led to a morphological and perhaps ecological difference. We are inclined to believe in the present case that the stage was set for subsequent differentiation and speciation by the single step of adding a chromosome to the genome of *australis*.

The close relationship of the genomes of *C. lingulata* and *australis*, the geographical juxtaposition the two taxa, and the restricted distribution of the former, suggests to us not only a rapid but also a relatively recent origin of *C. lingulata* from *C. biloba australis*.

Summary

Clarkia lingulata has been compared phenotypically and cytogenetically with each of the three subspecies of the closely related species *C. biloba*.

Clarkia lingulata is distinguishable in external morphology from *C. biloba australis*, which grows adjacent to it, only by the shape of the petal. Their genomes differ quantitatively by a chromosome, and structurally by a translocation and a paracentric inversion. Fertility of the hybrid between them is low, a consequence of the meiotic behavior associated with heterozygoscity for the translocation and the additional chromosome. The morphological difference is apparently also associated with this additional chromosome.

The genomes of *C. biloba australis* and *C. b. biloba* are structurally homologous. Consequently, their hybrids with *C. lingulata* are comparable in fertility. However, *C. b. biloba* differs to a greater extent from *C. lingulata* in external morphology.

Clarkia biloba brandegeae, which is geographically most remote from *C. lingulata*, shows the greatest difference both in chromosome arrangement and external morphology. Nevertheless, the hybrids between *C. lingulata* and *C. b. brandegeae* are more fertile than those involving the other two subspecies. The explanation lies in the more simple meiotic configurations resulting from a greater structural difference in the chromosomes. This explanation is substaniated by the fertility of the B_1 and subsequent progenies.

The conclusion is reached that *C. lingulata* evolved in comparatively recent time from *C. biloba australis* by a rapid process whereby a chromosome homologous with parts of two others was added to the genome of the latter.

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FECUNDITY OF HYBRIDS BETWEEN GEOGRAPHIC POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

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INTRODUCTION

Experiments on the viability of populations of *Drosophila pseudoobscura* from different localities in the western United States, and of F_1 and F_2 hybrids between these populations, have been described in previous publications (Vetukhiv, 1953, 1954). In these experiments certain numbers of young larvae of a given population are introduced in vials with a constant small amount of food, and the proportions of larvae which yield adult flies are used as a measure of the viability under competition. Now, the F_1 hybrids between the populations were, in six out of the seven cases tried, superior in viability to both parents, and in one case intermediate between the parents. This apparent heterosis does not, however, extend beyond the F_1 generation; F_2 larvae have viabilities intermediate between the parents, or, even, inferior to either parent. Similar experiments on *D. willistoni* and *D. paulistorum* showed that the degree of heterosis in hybrids between geographic populations varies from species to species; in *D. paulistorum* the F_1 hybrids were significantly superior to the parents in only one of the three crosses made, while the F_2 hybrids did not differ significantly from the parents.

In all of the above experiments, larval survival on a limiting amount of food was the only characteristic studied, and it remained questionable whether the apparent heterosis extended to other components of the adaptive value of the populations. After all, many sterile hybrids between species of plants are luxuriant as far as their growth is concerned, but the adaptive value of the hybrid genotype is equal to zero because of sterility. Therefore, experiments have been undertaken to study the fecundity and the longevity of the hybrids between the same local population of *D. pseudoobscura* that were used in the previous work, as well as some additional ones. The present report is devoted to a description of the observations on fecundity.

MATERIAL AND METHODS

The initial material consisted of 16 strains derived from wild flies collected at Gunnison, Colorado; 13 strains from Ferron, Utah; 15 from Bryce National Park, Utah; 10 from Mather, California and 6 from Piñon Flats, California. Each strain is descended from a single impregnated wild female. All strains were homozygous for the Arrowhead gene arrangement in the third chromosome, except that one of the Piñon strains was found, too late to be eliminated from the experiments, to be contaminated with the Standard gene arrangement. Most of these strains were used also in the previous experiments (Vetukhiv, 1953, 1954).

The flies whose fecundity was to be studied were obtained as follows. Virgin females were taken from approximately one-half of the strains to be crossed, and were placed together with males from the other half of the strains. Reciprocal crosses were also made, using males from the first and females from the second group of strains. This was done equally for the intra-locality crosses (referred to as P generation, or the control flies), and the inter-locality crosses (the F_1 and the F_2 generations). All the females, the fecundity of which was to be studied, were, thus, heterozygotes for genes and chromosomes derived from different wild

progenitors from the same or from different populations.

From each pair of reciprocal crosses 20 females and 20 males were taken and placed in 4 glass vials containing paper spoons with the cream-of-wheat and molasses medium. A drop of freshly made suspension of Fleischmann's yeast was placed on each spoon. Each vial had, therefore, 5 females and 5 males at the start of the experiments. With 5 intrapopulational (P) crosses, this means a total of 100 females; in the 10 interpopulational (F_1 or F_2) crosses, the total was 200 females. The control crosses were made twice, first simultaneously with the F_1, and second simultaneously with the F_2 crosses.

The spoons with the food were changed daily in all vials. The vials were kept in a constantly lit room with a temperature of 25° C. Under these conditions the mean duration of life of a female was between 40 and 50 days in the different crosses and generations. Two females lived 111 days each. The numbers of eggs deposited on each spoon were counted under a binocular microscope soon after the withdrawal of the spoons from the vials. The total number of eggs counted in the whole experiment was 405,081.

Comparison of Mean Values for Different Crosses

The data are reported in table 1 and in figures 1 and 2 in two ways. First, the mean numbers of eggs deposited per day per surviving female of a given kind are calculated. The oviposition has, of course, varied greatly not only in different vials, but also in the same vial on different days. Secondly, the total number of eggs deposited in a given vial, divided by the number of females (5), gives the number of eggs deposited by a female in her lifetime. All the F_1 interpopulational hybrids were tested simultaneously; the F_2 hybrids were tested some two months later but also simultaneously; control populations were tested together with the F_1 and also together with the F_2.

The mean number of eggs deposited in a day may be considered first (table 1, fig. 1). Of the 10 F_1 hybrid populations, 7 populations showed higher oviposition rates than either parental population; one F_1 hybrid (Gunnison × Ferron) appears

Table 1. *Numbers of eggs per female per laying day and per life in the F_1 and the F_2 hybrids and in the control crosses (P)*

	Per day P	Per life P	Per day P	Per life P
Mather	17.02	732.52	14.83	637.05
Bryce	15.10	574.45	16.58	811.55
Gunnison	15.45	709.20	16.74	643.50
Ferron	12.73	551.00	18.32	736.95
Piñon	14.71	643.65	15.55	566.95
Mean	15.20	642.10	16.50	679.20
	F_1	F_1	F_2	F_2
Mather × Bryce	16.30	776.95	18.65	718.60
Bryce × Gunnison	15.45	958.45	16.13	584.80
Mather × Gunnison	20.64	836.65	13.20	421.35
Mather × Ferron	18.35	787.55	14.65	620.15
Bryce × Ferron	21.04	1054.75	13.26	516.20
Gunnison × Ferron	15.89	649.95	16.60	676.35
Mather × Piñon	14.33	645.70	17.55	685.45
Bryce × Piñon	20.70	959.95	13.61	406.65
Gunnison × Piñon	19.40	502.05	12.48	315.90
Ferron × Piñon	21.83	934.40	17.35	637.55
Mean	19.00	810.50	15.60	553.30

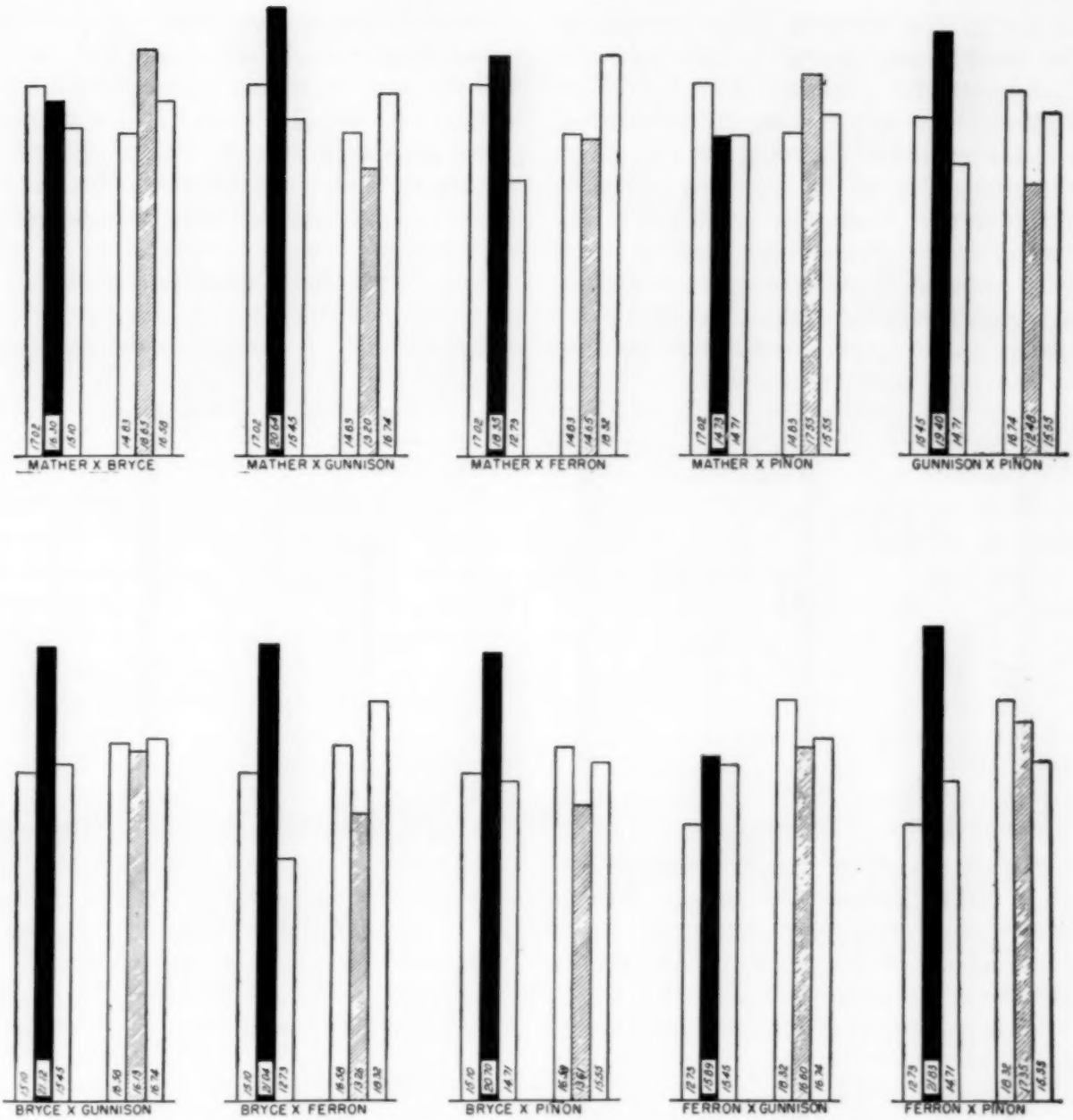

FIG. 1. Fecundity of geographic populations of *Drosophila pseudoobscura* and of hybrids between them.

The white columns symbolize the parental populations, the black columns—the F_1 hybrids and the cross-hatched columns—the F_2 hybrids between their populations. In every experiment the parental populations were tested simultaneously with the F_1 hybrids and re-tested with F_2 hybrids. The numerals indicate the mean of eggs deposited per day per female.

to be approximately as fecund as the more fecund parent (Gunnison); and two F_1 hybrids (Mather × Bryce and Mather × Piñon) are intermediate between the two parents. The F_2 hybrids fail to inherit the high fecundity of the F_1. In 7 out of 10 F_2 hybrids the fecundity is as low as or lower than in the least fecund parent; in one (Ferron × Piñon) the F_2 fecundity is intermediate; and two F_2 hybrids (Mather × Bryce and Mather × Piñon) are equal or superior in fecundity to both parental populations. It should be noted that the exceptional results in F_1 and in F_2 interpopulational hybrids are produced by the same two crosses; the crosses Mather × Bryce and Mather × Piñon show no superior fertility in the F_1 generation, but also no breakdown in F_2.

The numbers of eggs deposited by a

female in her lifetime show essentially the same thing (table 1 and fig. 2). Among the F_1's, seven out of ten show a greater fecundity than either parent, one (Gunnison × Ferron) an ostensibly intermediate fecundity and two (Mather × Piñon and Gunnison × Piñon) a fecundity as low as or lower than the parents. In the F_2 hybrids the conditions are again reversed. Among the 10 F_2's, 6 are apparently less fecund than the low parent, 3 are intermediate (Mather × Bryce, Gunnison × Ferron, and Ferron × Piñon) and 1 is superior (Mather × Piñon). It may be noted that the exceptional results in both F_1 and F_2 generations tend to be produced in the same crosses (Mather × Piñon, Gunnison × Ferron).

The evaluation of the statistical significance of the differences observed involves complications. As stated in the descrip-

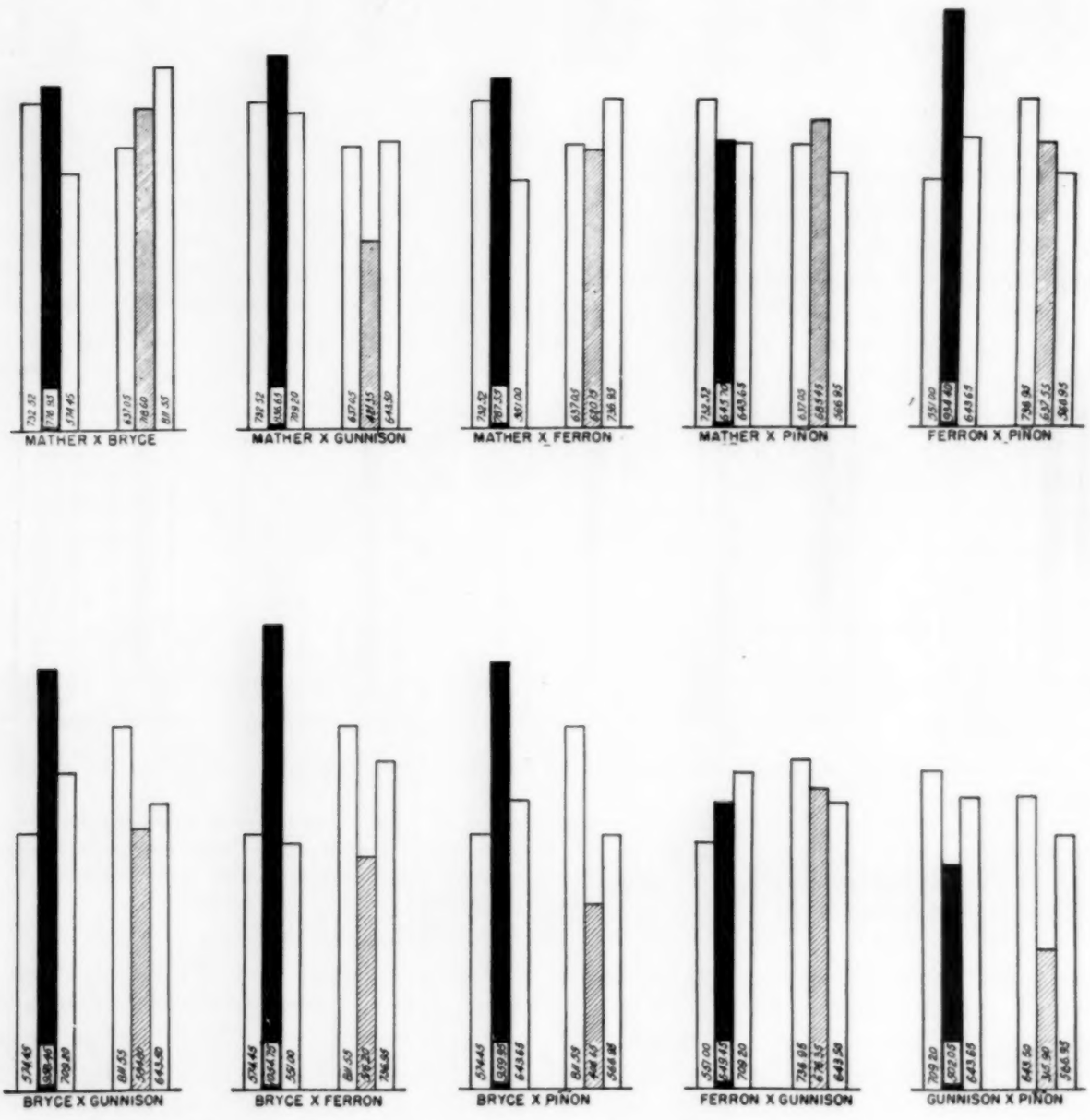

FIG. 2. Fecundity of geographic populations of *Drosophila pseudoobscura* and of hybrids between them.

The white columns symbolize the parental populations, the black columns—the F_1 hybrids and the cross-hatched columns—the F_2 hybrids between their populations. In every experiment the parental populations were tested simultaneously with the F_1 hybrids and re-tested with F_2 hybrids. The numerals indicate the mean of the eggs deposited per life per female.

tion of Methods, every type of fly was tested for fecundity by having 4 vials, each initially with 5 females and 5 males. The results obtained in the different vials naturally varied, and it becomes important to demonstrate that the differences between the crosses cannot be accounted for by the different behavior of the flies in different vials. Professor Howard Levene has very kindly made the necessary analysis.

Statistical Analysis

(by Howard Levene)

Since there are four replicate values for each cross, it is possible to test whether there is significant heterogeneity between the strains from different localities or the crosses between different localities. For the number of eggs per laying day, the difference between the ten different crosses gives F values of 3.87 and 3.45 for the F_1 and the F_2, which are significant at the 1% level. The F values for the difference between the five parental strains are 3.61 for the F_1 control, which is significant at the 5% level, and 0.76 for the F_2 control which is not significant.

For the number of eggs per female over her total lifetime, on the other hand, only F_1 gives an F value significant at the 5% level. Because strains of flies from different localities might be expected to behave differently and did because the F tests bore this out, for the data on eggs per laying day at least, it was felt that any general conclusions on the behavior of F_1 and F_2 generations from inter-locality crosses should be based on the fact that we had a sample of 5 observations (i.e., five localities). Accordingly, tests were performed using the means of all determinations on a given strain or cross as the basic data. The results are given in table 2. For the number of eggs per laying day, there were significant differences between the F_1 and F_2 and between the F_1 and the control values for the parental strains, but not between the two sets of control values or between the F_2 and its control. On the other hand, for the number of eggs laid per lifetime, only the difference between F_1 and F_2 is significant, although the differences between the F_1 and the control, and the F_2 and the control, are close to significance (P less than 10%). We can thus conclude that for both measures of egg laying the F_1 is superior to the parental strains, or the F_2 is inferior to the F_1, but that it is uncertain whether the F_2 is inferior to the parental strains. Finally, there is no apparent difference between the two sets of measure-

Table 2. *Comparison of the mean values for the different crosses. P_1 and P_2 refer to the control populations examined simultaneously with the F_1 and the F_2 interpopulational crosses respectively*

Comparison	Mean values	Difference	Standard deviation	t	Degrees of freedom	Significance (%)
			Eggs per Life			
P_1–P_2	642–679	− 37	71.9	0.52	4	—
F_1–F_2	811–558	252	69.0	3.66	9	0.5
P_1–F_1	642–811	169	82.6	2.04	13	6
P_2–F_2	679–558	−121	68.8	1.82	13	3
			Eggs per Day			
P_1–P_2	15.0–16.4	− 1.4	1.2	1.13	4	—
F_1–F_2	19.0–15.4	3.6	1.3	2.80	9	2
P_1–F_1	15.0–19.0	4.0	1.2	3.32	13	0.6
P_2–F_2	16.4–15.4	1.0	1.1	0.97	13	—

TABLE 3. *Values of T for the differences between the control (P) and the F_1 and the F_2 hybrids*

	Eggs per Lifetime					
	Mather	Bryce	Gunnison	Ferron	Piñon	
Mather		1.22	1.10	1.54	0.006	
Bryce	0.24		3.48*	2.84*	2.71*	F_1—control
Gunnison	−2.53*	−0.59		−0.08	−1.27	
Ferron	− .38	−1.90	0.31		3.13*	
Piñon	0.28	−2.83*	−5.77*	0.18		
			F_2—control			

	Eggs per Laying Day					
	Mather	Bryce	Gunnison	Ferron	Piñon	
Mather		0.28	3.48*	−0.81	3.23*	
Bryce	1.81		4.39*	2.82*	5.30*	F_1—control
Gunnison	−2.15	0.89		1.40	0.11	
Ferron	1.97	−1.75	−2.23		5.22*	
Piñon	−0.90	−1.58	0.69	1.40		
			F_2—control			

* Value of t significant at 5% level.

ments on the parental strains done as controls at the time of F_1 and at the time of the F_2 crosses.

Since the over-all comparisons of the F_1 with the controls and the F_2 with the controls were inconclusive, it was decided to make the comparisons separately for each cross. For this purpose the mean for a given cross was compared with the average of the means for the two parental strains. Since the values found at different times had proved comparable, the two sets of values for each parental strain were combined. Because it was felt that the variances for the parental strains and for the hybrids might differ, the method of Welch (1949) was used for computing t and for testing its significance. The results are given in table 3. For eggs per laying day, the mean for 9 out of the 10 F_1 crosses was greater than the average of the parental means, and 6 of the 9 were significantly greater. For the F_2, on the other hand, 5 values were greater and 5 less than the parental average, and none were significant. For eggs per lifetime, 8 out of 10 F_1 crosses gave higher values, although only two of these were significant. Six of the F_2 crosses were worse than the average of the parental strains, 2 being significant, and 2 other t values being close to significance, while all 4 positive t values were small.

The general picture is thus that the F_1 is often, but not always, superior to the mean of the parental strains for both measures of fecundity, while the F_2 may be inferior to the parental strains with respect to eggs per total lifetime, but is es-

sentially equivalent to them for eggs per laying day.

Discussion

Maynard Smith and Maynard Smith (1954) concluded that "the greater biochemical versatility to be expected in a heterozygous organism may well confer greater fitness. If so, the recovery of vigor on crossing inbred lines may not be simply a matter of covering up deleterious recessives, but may also be due to the fact that at the same loci the heterozygote is fitter than either homozygote. . . . Large size, high growth rate and good viability can reasonably be regarded as evidence of the greater potentiality of heterozygous organisms." This statement may be said to describe the situation in the interpopulational hybrids in *Drosophila pseudoobscura* (Vetukhiv, 1953, 1954). These populations themselves are large and flourishing and their replicas made in the laboratory by intercrossing several wild strains can in no sense be regarded as inbred. Nevertheless, the F_1 hybrids between the populations show even greater vigor, not only with respect to the competitive ability of the larvae but also in the adaptively so important a trait as fecundity of the hybrid females. Although the over-all adaptive values of the geographical populations and their hybrids are still incompletely known, it seems at present justified to describe the F_1 hybrids between the population as heterotic. Brncic (1954) discovered that heterotic effects occur not only when populations themselves are crossed but also when a chromosome from one population is made heterozygous on the genetic background of another population.

Dobzhansky, Pavlovsky, Spassky and Spassky (in press) have shown that heterozygotes carrying pairs of chromosomes from the same natural population of *Drosophila pseudoobscura* are not necessarily superior to homozygotes in all environments. Though rarely, some homozygotes are supervital in some one environment. But while heterozygotes show a superior homeostatic adjustment and remain highly viable when the environment is varied, the homozygotes are narrowly specialized and suffer when placed in different environments. An important fact is, however, that the heterozygotes which carry two different chromosomes both of which are subvital when homozygous are neither more nor less viable than the heterozygous. The importance of the homeostatic adjustment in the production of heterosis has also been stressed by Lerner (1954). Stone, Alexander, and Clayton (1954) showed that the amount of heterosis in hybrids between geographic populations varies from species to species. They crossed strains of *D. novamexicana,* which is a rare species, and also of *D. hydei* taken from a region in which this species is uncommon. A barely perceptible heterosis was observed, restricted in *D. novamexicana* to a somewhat greater survival of hybrid larvae; no heterosis appeared with respect to fecundity. Robertson and Reeve (1955) intercrossed strains of *D. melanogaster* which were selected for large or for small body size, and also unselected strains. They found that intercrossing of small strains results in a striking heterosis, while crosses between large strains gave intermediate F_1 hybrids. Examination of figures 1 and 2 shows that in our material heterosis was also not an invariable rule (see also Vetukhiv, 1954). Genetic situations which lead to heterosis in intercrosses between populations remain to be determined.

Moreover the heterosis exhibited by the F_1 interpopulational hybrids in *D. pseudoobscura* is not inherited by the F_2. As a matter of fact, there is a good basis to suspect that the F_2 of many crosses suffers a genetic breakdown, and is less vigorous not only than the F_1 but even than the parental populations. Such a breakdown was observed by Brncic (1954), when a chromosome of geographically foreign origin was broken up by crossing over. Wallace (1955) has obtained most enlightening results by comparing the via-

bilities of larvae from certain populations of *D. melanogaster* and of hybrids between them. He was able to obtain hybrids which had different degrees of heterozygosity, and also different proportions or "original" and of "derived" chromosomes. "Original" chromosomes are intact chromosomes coming from the respective populations, and "derived" ones are compounded by crossing over of sections of chromosomes from different populations. Wallace found that heterozygosity and possession of "original" chromosomes and chromosome sets are conducive to heterosis, while homozygosity and "derived" chromosomes tend towards breakdowns of viability. The work of King (1955) has yielded another evidence of genetic breakdown in the F_2 hybrids between populations; in this case, two lines of *D. melanogaster* selected for a resistance to DDT have produced resistant F_1 hybrids, but an F_2 with a weakened resistance.

As pointed out in a previous publication (Vetukhiv, 1954), the breakdown of heterosis in F_2 hybrids between geographically different populations shows that the gene pool of each population is a coherent adaptive system evolved under the control of natural selection. The fact that the F_1 hybrids between such populations are heterotic may appear strange. Why, indeed, should natural populations not exploit the possibilities of adaptive betterment which are easily available through hybridization? The breakdown in F_2 supplies is a possible answer: The heterosis observed in the F_1 generation is not inherited by the future hybrid generations.

Summary

The fecundity has been studied in populations of *Drosophila pseudoobscura* from six localities in the western United States, and in the F_1 and F_2 hybrids between these populations. The fecundity was measured either as the mean number of eggs deposited by a female per day or as the mean total number of eggs per female per lifetime. With both measures the F_1 hybrids are mostly superior to both parental populations. The F_2 hybrids do not inherit their heterosis, and in some cases their fecundity is even lower than that of the parents.

Acknowledgments

The author wishes to acknowledge his obligations to Professor Th. Dobzhansky for his interest and for his kindness and advice, and to Professor Howard Levene for his help with statistical analysis.

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AN ANALYSIS OF VARIATION IN A VARIABLE POPULATION OF CLADONIA

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Fairly early in his studies of variation in natural populations, the senior author realized that he apparently could detect the effects of hybridization in the field even for organisms with which he was unfamiliar. When the suspicion first arose that this might indeed be so, he dismissed it as fantastic. Not until this intuitive judgment had been repeatedly confirmed by the most rigorous experimental tests did he start looking for the objective facts behind the intuition. It was then readily determined that populations which suggested hybridization to the experienced eye had much the same variation pattern as first and second back-cross populations in experimentally produced hybrids. The phenomenon was eventually discussed in print under the title of "Concordant versus discordant variation" (Anderson, 1951) and illustrated by examples which, though hypothetical, were closely modeled upon experimental data (Anderson and Gage, 1952).

The analysis of variation in Cladonia presented below is one of several attempts to test the hypothesis that introgression can be recognized by an experienced observer. The population was discovered by the senior author. Though he knew nothing about the morphology or classification of lichens, the look of entire populations was strikingly "discordant". It presented to the observer that criss-crossing of several variables which is characteristic of introgression whether studied in the field or in the experimental plot. To the senior author it appeared as if two species of lichens had hybridized and the hybrids had back-crossed once or twice to the parental species. The impression was not of a hybrid swarm but of that heightened variability in the participating species which is characteristic of introgression. Accordingly he prevailed upon a trained lichenologist to undertake a joint investigation of this population. It will be seen that the results confirm and extend the working hypothesis. That one was able to make successful predictions of this sort in advance of any scientific literature on the subject, in a group of organisms with which he was wholly unfamiliar suggests that there may be more fundamental similarities in the nature of species in different groups of organisms than we have dared to suspect. If a scientist accustomed to working with hybridizing populations of flowering plants, can recognize a similar situation in lichens, even when he knows nothing about lichens, speciation in lichens and speciation in flowering plants must have certain fundamentals in common.

The markedly "discordant" populations of Cladonia were found a few miles southeast of Pacific, Missouri, in the La Barque Creek area. In ravines at the junction of the St. Peter's sandstone and the Joachim limestone were large Cladonia mats. Within a single mat there usually appeared to be numbers of plants belonging to two distinct species, *C. subtenuis* (des Abbayes) Evans and *C. uncialis* (L.) Web., of different subgenera, with those of each species showing wide plant-to-plant variation. A comparative study of these two species seemed valuable because of what it might tell about the extent of variation not only within each species but also within the genus. The pictorialized scatter diagram (Anderson,

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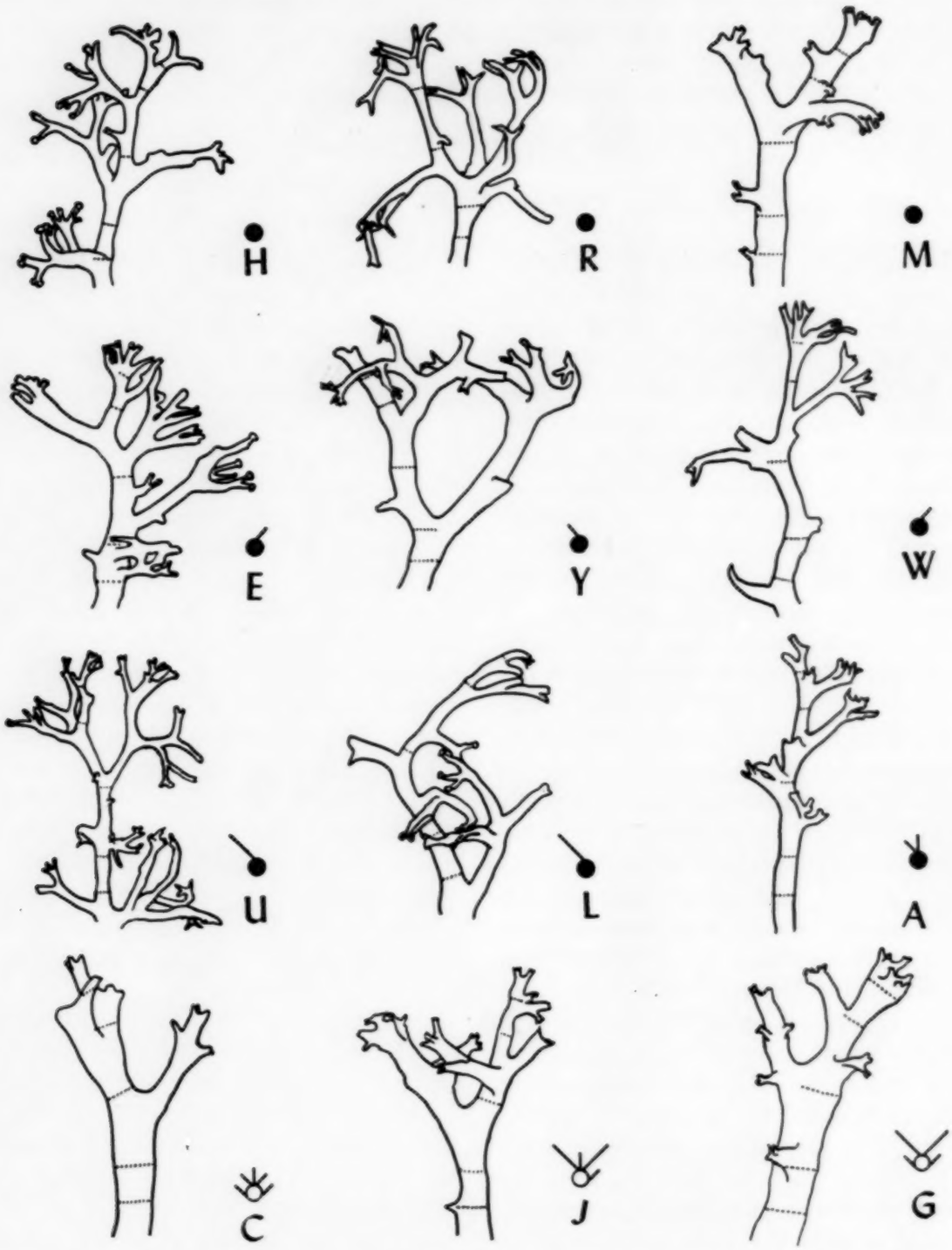


FIGS. 1 and 2. Camera lucida drawings of the ultimate centimeter of a podetium from each specimen studied. Dotted lines indicate distances ⅛, ¼, ½, ¾ and ⅞ of the distance from the tip. Glyphs indicate scores for characters as given in key of figure 7; additional lower rays to left and right represent abscissa and ordinate characters, respectively (left: no ray, .2–.5 mm.; medium ray, .6–.9 mm.; long ray, 1.0 mm. and up; right: no ray, .2–.5 mm.; medium ray, .6–.7 mm.; long ray, .8 mm. and up). The drawings have been arranged according to the total index value of each specimen as shown in figure 8. As in figures 7 and 8, the color of the center of the glyph indicates the taxonomic determinations made by Dr. Evans. Black represents *C. subtenuis;* white, *C. uncialis;* stippled, *C. uncialis obtusata.*

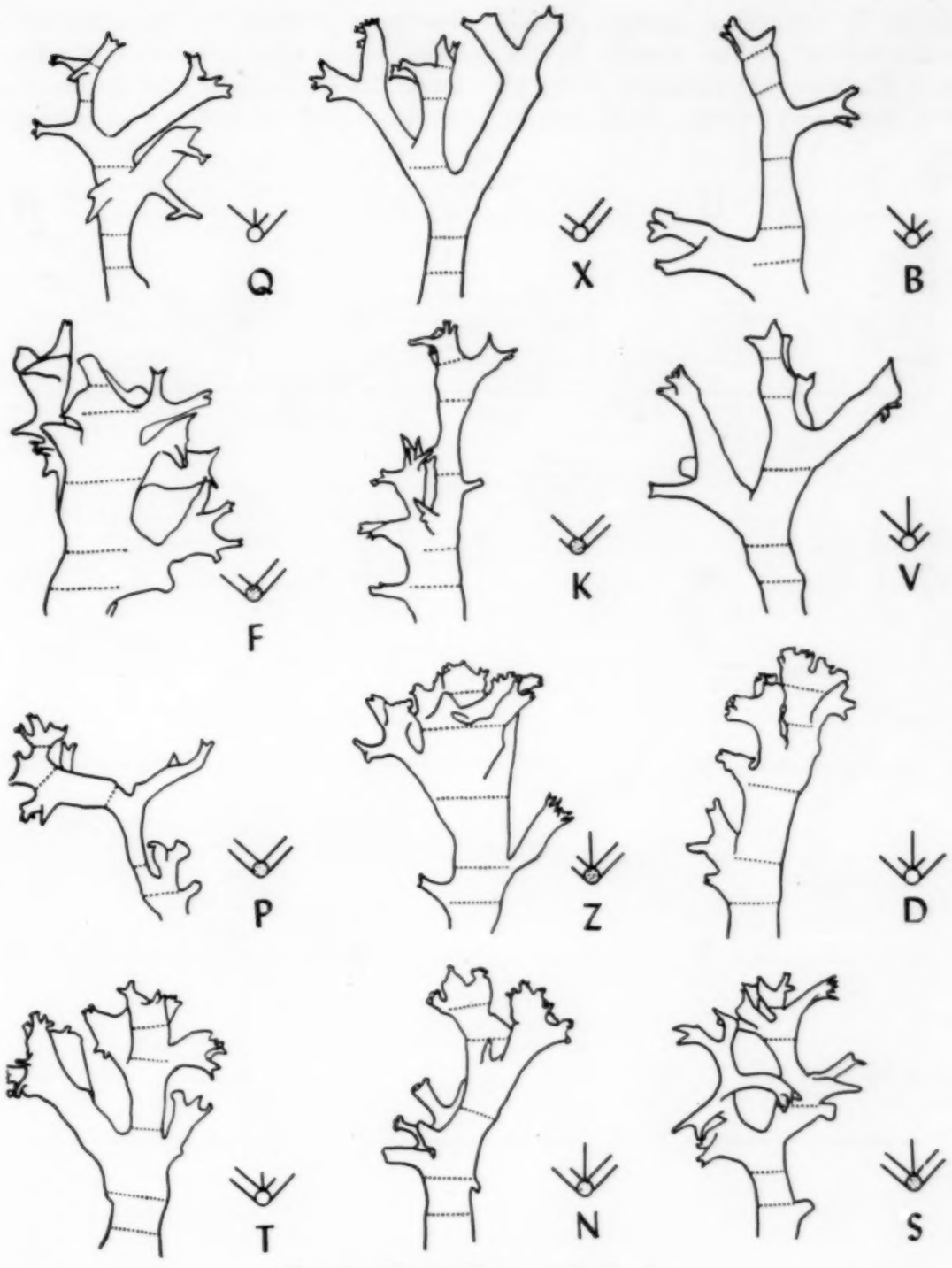


FIG. 2. For caption, see figure 1.

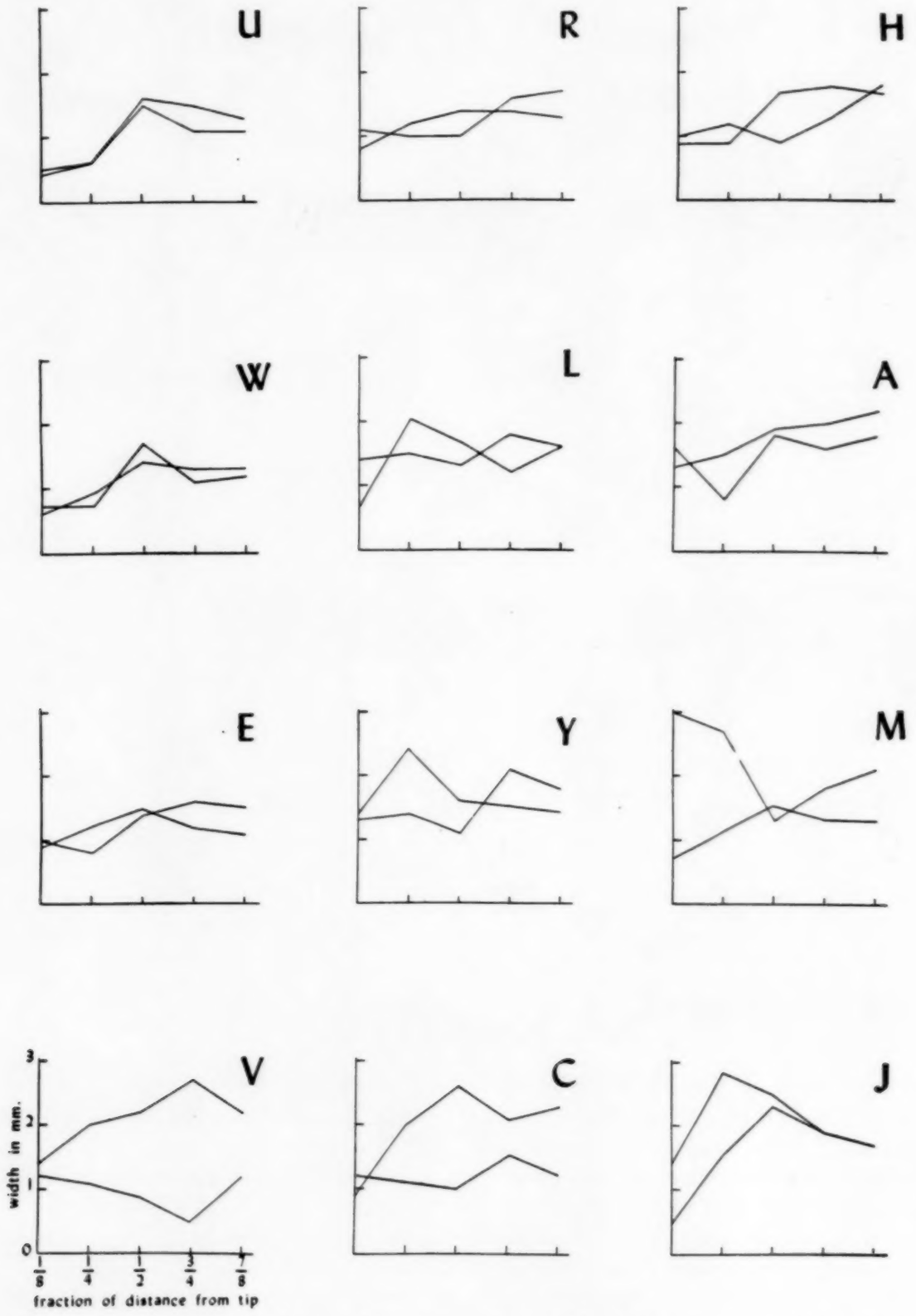
1949) has been used successfully in variation analyses of flowering plants for the comparison of individuals considering more than two characters at one time; it was therefore applied to this problem.

From a single large mat, 24 distinct uniform masses assumed to be individual plants were selected at random. The specimens were all pressed in the same manner. It is of interest to note that since all of the collections were made from a single mat there should not be great habitat differences.

The following characters were compared.

1. *Index of variation in width within the ultimate podetial centimeter.* The best measurable difference between the two species studied was found to be the index of variation in podetial width (the

differences in branching pattern which are characteristic of the species in the subgenus Cladina, des Abbayes 1939, to which *C. subtenuis* belongs could not be used as a comparative character since the branching is of a different type than that found in *C. uncialis*, sub-genus Coenomyce). Such an index was obtained by



FIGS. 3 and 4. Graphs giving relationships of podetial width measurements for the two tips of each specimen. Letters correspond to those of figure 1.

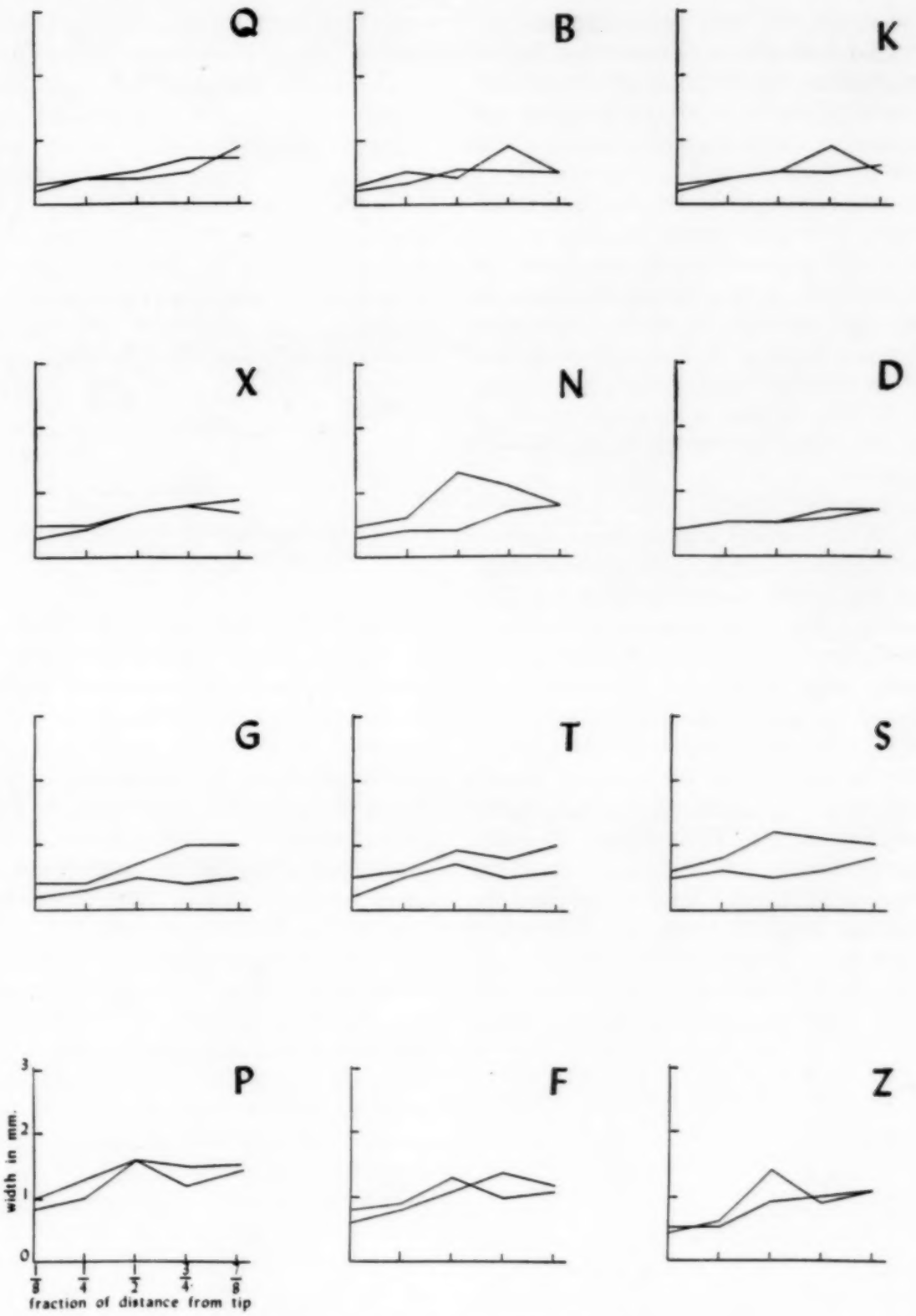


FIG. 4. For caption, see figure 3.

(a) making camera lucida drawings of the ultimate centimeter of two podetia of each specimen, (b) measuring the width on the drawing of each segment at points 1/8, 1/4, 1/2, 3/4, and 7/8 of the distance from the tip and reducing to actual size (to nearest .1 mm), (c) subtracting the smallest measurement from the greatest measurement obtained for each segment to give a number indicating the degree of absolute variation in the width of the individual segments, and (d) calculating

the mean of the two such numbers obtained for each plant. These mean values were used as the ordinant in the scatter diagram. Camera lucida drawings of one podetial tip of each specimen are illustrated in figures 1 and 2. Graphs of the two sets of measurements for each specimen are shown in figures 3 and 4.

2. *Width of podetium ⅛ cm. from tip.* The average of the measurements (determined as in 1 above) made ⅛ cm. from tip in two podetia of each specimen was used for the abscissa.

3. Width of the widest podetium of each specimen, measured to the nearest millimeter.

4. Cortical index. For microscopic study of the cortical region (in *C. uncialis* a true cortex and in *C. subtenuis* the medullary hypae free and external to the algal colonies), free-hand cross-sections taken from the interstitial region of one mature podetial stalk from each specimen were mounted in lacto-phenol containing acid fuchsin. It was apparent from a study of the sections that the cortical region varied in two respects: (a) in the degree of erosion, and (b) in the degree of gelification of the hyphae. In some cases the hyphae were loosely interwoven and the individual hyphae could be determined because the walls were fused showing only occasional cell lumens (the gelified condition). In general the loosely interwoven hyphae were found in those cortical regions which were most eroded. The first of the two differences was measured by determining the difference between the extremes in width in each cross-section (width measured by micrometer eyepiece) giving a value directly related to the degree of erosion. A measure of the second was obtained by giving each specimen a score ranging from 0, loosely interwoven hyphae, to 2, extremely gelified. These two sets of comparisons were then combined in a scatter diagram. The resulting distribution more or less fell into 3 categories, (a) not eroded, heavily gelified; (b) variable; and (c) eroded, not gelified. These were then used as the cortical index in the pictorialized scatter diagram.

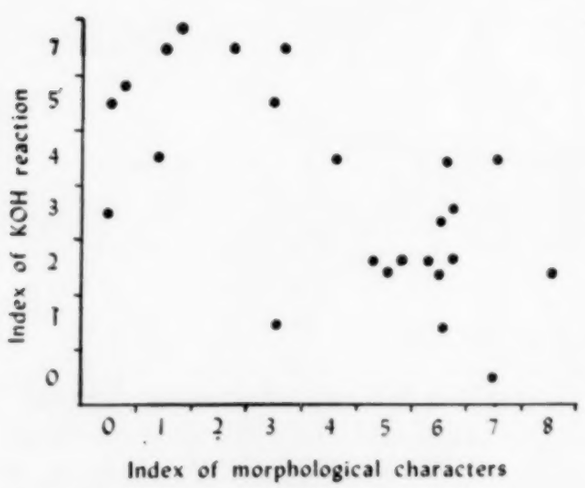


FIG. 5. Scatter diagram showing correlation of KOH reaction with index derived from four morphological characters.

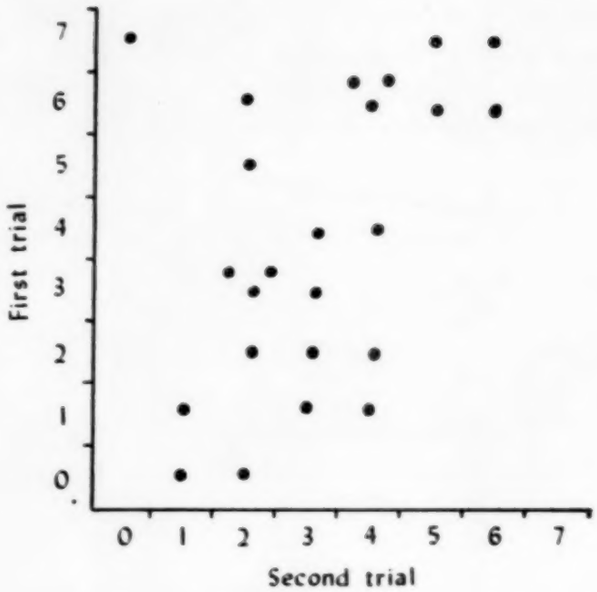


FIG. 6. Scatter diagram showing correlation of first and second KOH reactions.

5. *Reaction to KOH* (figures 5 and 6). A drop of 10 per cent KOH and a drop of tap water were placed in separate spots on the dry podetium. Readings scored from 0 (KOH drop no different from water) to 4 (a very apparent change to yellow in the KOH drop), were made immediately and again after five minutes and the two readings were totaled. A duplicate test was made and the two sets of scores averaged for the final scores. These final scores ranged from 0 to 6.

It should be noted that the strong yellow color reaction common in some Cladonia species was not found in either of the species used here. The measure is of a slighter color change, but one which is apparent when the comparison is made with a spot upon which only tap water was placed.

Of the characters compared the first three are measures of gross morphological variation, the fourth is an anatomical characteristic and the last a physiological reaction.

The twenty-four specimens were scored in the five ways described above and the results combined in the pictorialized scatter diagram (fig. 7). It is apparent that the two species represented, *C. subtenuis* and *C. uncialis,* separate well from each other on the scatter diagram. The same specimens, identified only by number, were sent to Dr. Alexander W. Evans for standard taxonomic determinations independent of those made by the junior author. Taxonomically, *C. uncialis* is subdivided into numerous formae. Ac-

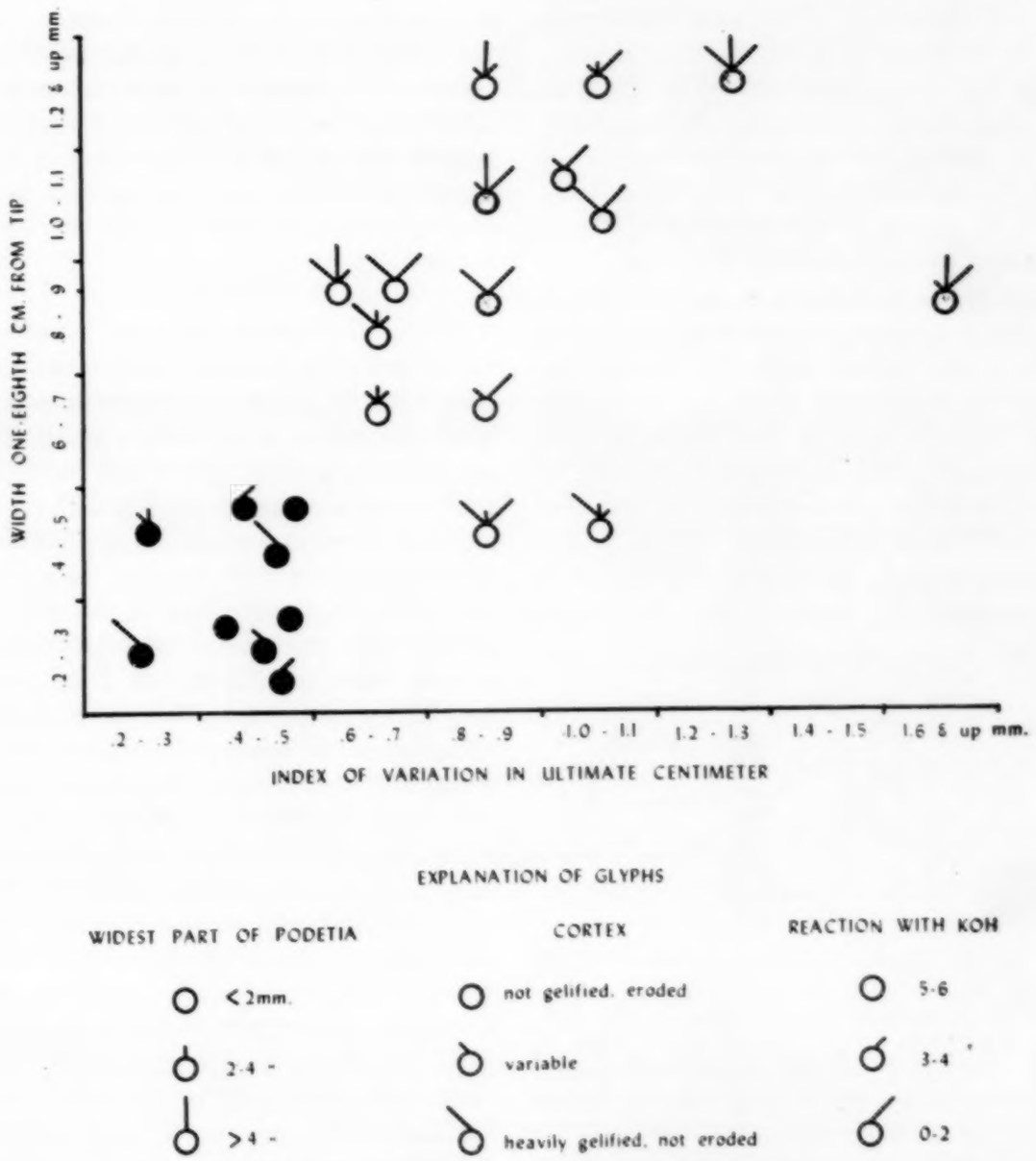


FIG. 7. Pictorialized scatter diagram of Cladonia population (24 samples). Shading of glyphs denotes identification according to Evans. As in figures 1, 2, and 8, black represents *C. subtenuis;* white, *C. uncialis;* stippled, *C. uncialis* f. *obtusata.* See text for explanation of the scoring.

cording to Evans, some of the plants in this study (those appearing in the scatter diagram as the most extreme in both index of variation in podetial width and in width ⅛ cm. from tip) could be referred to forma *obtusata.*

In figure 8 the data of figure 7 have been condensed into a five-fold index. The two characters measured on the horizontal and vertical axes and the three characters diagrammed on the rays have each been scored in three grades, 0, 1, and 2. The three rayless glyphs in the lower left hand corner of figure 7 represent one extreme. The score for each of them is 0 for each of the five characters giving them index values of 0. The other extreme is represented by the specimen in the upper right hand corner of figure 7, the only glyph with three long rays. It scores 2 for each of the five characters giving it a total index of 10.

Figures 7 and 8 (and most particularly the latter) serve as a convenient way to compare our results with Dr. Evans' independent judgments from the same material. It will be seen that there is close agreement. Our scale gives values of from 0 to 3 for all the plants determined as *C. subtenuis* while none of the plants determined as *C. uncialis* scores below 5. Furthermore, *C. uncialis* and its forma *obtusata* have overlapping values, *C. uncialis* from 5 to 8 and forma *obtusata* from 7 to 10. For this population, as seen against the background of various studies of introgression, we would advance as a very likely hypothesis the notion that its ultimate origin was from extreme forms of *C. subtenuis* and *C. uncialis* f. *obtusata.* Hybrids between these two lichens which crossed back to the parental extremes then produced at first or second hand the high-scoring variants of *C. subtenuis* (with index values of 1 to 3) and all the *C. uncialis* which are not in forma *obtusata,* perhaps even the lowest scoring plants of that forma. As in the various other field studies of hybridization which have been undertaken in this laboratory the chief result of hybridization seems to be that greatly enhanced variation in the parental species is presented to natural selection to work upon.

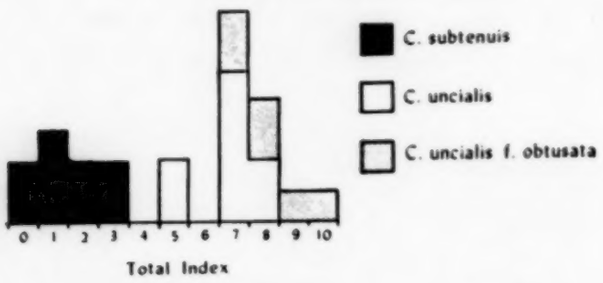


FIG. 8. Frequency distribution of the Cladonia population based on the total index of scores obtained from the pictorialized scatter diagram (fig. 7) by totaling the ray values for each glyph (ordinate and abscissa characters are represented by rays as described in fig. 1 and 2). For each of the five characters, a low value (no ray) is scored 0; a medium value (short ray) is scored 1; and a high value (long ray) is scored 2.

DISCUSSION

The utilization of the pictorialized scatter diagram in studying variation in these two Cladonia species seems significant. With the use of even such a small sample as 24 specimens, by using correlated comparative characteristics, the relative amount of variation can be approximately ascertained. It also permits the comparison of many types of variation, i.e., morphological, anatomical, and physiological at one time. Primarily, it facilitates the graphic measurement of morphological differences which previously have been compared intuitively, e.g., the relative difference in taper of the podetia. The variation found in this study has certain biological implications. Vainie (1897) who has made the most complete study of the genus yet published believes that the species variation found is due mainly to external conditions and not to internal genetic variation. He was of the opinion that sexual reproduction was entirely lacking in this genus. Recent studies establishing sexual reproduction in a related genus (Johnson 1954) indicates that it is also possible in Cladonia. The very common occurrence of spermagonia and

apothecia in Cladonia would of itself indicate sexuality as would also the production of viable ascospores in large numbers (Rudolph, unpublished data). Although it is felt by most lichenologists that various methods of asexual reproduction are much more important in lichen multiplication than is the sexual method, this need not rule out the rare successful sexually reproduced offspring which is all that is necessary for genetic variability. Variation may also be due to heterocaryosis resulting from anastomosis of two mycelial filaments occurring in a manner similar to that found in Neurospora and other Ascomycetes. It is apparent that the large amount of variation in some species could not be produced by habitat variation alone. This study of plants growing in the same mat in an apparently uniform habitat demonstrates considerable variation which could not be due to differences in age, particularly since only mature specimens were studied.

The fact that the variation pattern of this population is so similar to that met with in colonies of the higher plants in which there had been hybridization and consequent back-crossing, suggests not only that there is sexual reproduction in these lichens but that there is introgression. The variation within *C. subtenuis* is all in the direction of *C. uncialis*. The variation in *C. uncialis* is all in the direction of *C. subtenuis*. Introgression is the only known evolutionary force which produces population variation patterns of this kind (Anderson, 1949). There is strong presumptive evidence that the extremely variable population in the La Barque Creek area came originally from hybridization between *C. uncialis* and *C. subtenuis;* that the primary hybrids crossed back to each of the two parental species, thus producing for each species a group of variant forms within which the plant-to-plant variation for one or more characters forms a gradient in the direction of the other species. Cladonia is a notoriously variable and taxonomically difficult genus. The studies here reported suggest that hybridization may well be one of the prime factors responsible for this confusion.

Summary

1. A strongly discordant variation pattern brought large variable populations of Cladonia to our attention.

2. Random collections were made from an essentially uniform habitat and the variation pattern was studied intensively

3. Five characters were eventually chosen for scoring or measurement:

(a) variability in width in the ultimate podetial centimeter.
(b) podetial width $\frac{1}{8}$ centimeter from tip.
(c) maximum podetial width.
(d) erosion and gelification of the cortical region.
(e) color reaction to KOH.

4. These five characters were found to be associated in two complexes running from:

little variation in width in podetium tip
distal region of podetium narrow
podetium narrow
cortical region not gelified, eroded
no reaction with KOH

to:

great variation in podetium tip
distal region of podetium wide
podetium wide
cortical region heavily gelified, not eroded
yellow reaction with KOH

5. Analysis by pictorialized scatter diagrams and by the use of the hybrid index method, correlated closely with the judgment of a taxonomic expert, made independently on the same material.

6. The probable sexuality of these species of Cladonia is briefly discussed. It is concluded that they give evidence of sexual as well as asexual reproduction. It furthermore seems most likely that hybridization and subsequent backcrossing between *Cladonia uncialis* and *C. sub-*

tenuis are responsible for the extreme variability of this population.

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THE GEOGRAPHIC HISTORY OF *QUERCUS AJOENSIS*

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INTRODUCTION

Amongst the sources of data in historical plant geography, the principal source is that great group of phenomena that indicate geographic distribution of species in earlier times. Fossils of all kinds, written records from early historical periods, relictual stands, and evidences of hybridity beyond the present range of a parent species all furnish factual information concerning previous distributional patterns. Relictual stands and hybrids are particularly valuable because of their trustworthy characters. The genetic material they indicate is still present for identification in the living state; they require less acceptance "on faith" than do historical records and provide more certain (and confirmable) identification than do fossils.

In this study there is attempted a reconstruction of a portion of the biological history of *Quercus ajoensis,* a recently described species of the Ajo Mountains in southern Arizona. Although collected as early as 1933 (F. Shreve 6200, Alamo Canyon, Ajo Mts., Mar. 19, 1933; U. Ariz. Herbarium 49,177), it has been consistently identified as *Q. turbinella* Greene, a species to which it bears a superficial resemblance. Dr. A. R. Phillips (1948) appears to have been the first to question its conspecificity with the latter. Both of the present writers have in the past separately examined one or more specimens from the Ajo Mountains and reluctantly identified the species as *Q. turbinella.* The quality of the specimens available did not permit a complete characterization of the plant until Dr. K. F. Parker submitted to us a well chosen series of collections that fully established the distinctness of the species from *Q. turbinella.*

Subsequently, there came to our attention specimens originally taken as atypical *Q. turbinella,* bearing a marked resemblance to *Q. ajoensis.* Preliminary study of these specimens, from two low desert ranges, the Castle Dome and Kofa Mountains, in Yuma County, Arizona, indicated that they were essentially intermediate between *Q. turbinella* and *Q. ajoensis,* and therefore we take them to be of hybrid origin. Belatedly, we have learned of still a third hybrid locality, in the Eagle Tail Mountains of eastern Yuma County. We are indebted to Dr. Charles T. Mason, Jr. for sending us a collection (J. T. Wright No. 50–55, March 7, 1955) from this range, which is also distinctly intermediate in the sum of its characters.

Following two extensive joint field studies, *Q. ajoensis* was described by one of us (Muller, 1954) preliminary to the present paper.

It is our purpose to analyze the several lines of evidence available, to present a biometric analysis of the living populations of both parents and their hybrids, and to offer in support of this ecological, geographical, and paleontological evidence in an effort to establish a broad basis for reconstruction of at least a portion of the history of *Q. ajoensis.*

ECOLOGY AND GEOGRAPHY OF THE SPECIES

The range of *Q. ajoensis* is restricted to a few high canyons in the Ajo Mountains, Pima County, Arizona (see fig. 1). There exists a distinct probability that the species also occurs in some of the more prominent mountains of adjacent Sonora, Mexico, where botanical explorations have been meager at best. Save for

a single area in northeastern Pinal and southwestern Gila counties,[1] the northward extension of its present range seems unlikely. The mountains of adjacent areas in Arizona have not yielded it; in fact, these mountains are in general too low and too desiccated to harbor a tree of the nature of *Q. ajoensis*.

The position of the hybrid populations in Yuma County lies midway between the present range of *Q. ajoensis* and that of *Q. turbinella,* which is suggestive of a formerly more extensive range of *Q. ajoensis*. This is also supported by data derived from a detailed study of epidermal leaf characters. The fossil record, fragmentary though it is, has yielded uncommonly conclusive evidence in further support of this northward extension of *Q. ajoensis* in the geological past.

Since the two species under consideration usually grow in mountains well above the levels of meteorological observatories, it is not possible to delimit exactly their present ranges in terms of the temperature and rainfall values of their specific habitats. Furthermore, extrapolation from the nearest observatories, when it is performed repeatedly in different situations, is likely to yield erroneous results. Yet, the ranges of the species are sufficiently discrete so that a climatological control of this phenomenon is almost a certainty. Certain generalizations concerning their climatic tolerances may be derived from the present distribution and associates of the species.

Quercus ajoensis grows in moist canyon bottoms, on grassy slopes, and at the bases of high north-facing canyon walls in the Ajo Mountains where it occurs in "pure" form between 2800 and 4000 feet elevation. Its obvious reproduction here by seedlings is regarded as evidence that the climatic conditions fit its requirements currently. Its associates at the upper elevations are perennial grasses and scattered junipers. Because no more mesic trees occur here, the area can be classified only as grassland. Were the mountains sufficiently high to induce greater rainfall and lower temperatures, a greater variety and a greater density of woodland species might be expected to form a woodland community, but this does not develop anywhere in the Ajo Mountains.

At lower elevations dilute hybrid forms of *Q. ajoensis* mixed with relatively "pure" individuals occur in a streamside or canyon bottom woodland of *Forestiera phillyreoides* (Benth.) Torr., *Simmondsia californica* Nutt., *Juniperus deppeana* Steud., *Celtis reticulata* Torr., and *Vauquelinia californica* (Torr.) Sarg. Away from the streams a thorn-scrub vegetation, consisting mostly of *Prosopis juliflora* (Swartz) DC. and *Acacia greggii* Gray with scattered perennial grasses and cacti, forms the cover. Most of these associates are southerly in their affinities and none, except possibly the *Celtis,* is ever associated with the woodland of central and northern Arizona and of higher elevations.

The nearest meteorological observatory with a significant length of record (24 years) is at Ajo, about 35 miles north of the Ajo Mountains and at an elevation of 1850 feet (see table 1). This station has recorded an average annual rainfall of 9.14 inches, January average temperature of 52.0° F., and July average temperature of 89.4° F. Significantly, Quartzite, with a 24 year record, which lies nearest the hybrid populations in Yuma County, Arizona, at an elevation of 900 feet, has an average annual precipitation of 5.79 inches, a January average temperature of 49.7° F., and a July average temperature of 94.0° F. (Greening, 1941). In both instances the populations of *Q. ajoensis* and hybrids of this species lie at some

[1] A population sample collected in Pinal Co. after this manuscript had gone to press (H. S. Haskell and J. M. Tucker 2896-1 to 25, Oct. 1, 1955, Oak Flats Recreation Area, ca. 3½ miles east of Superior) includes a number of individuals which are essentially *Q. ajoensis*. Most of the population, in fact, approaches this species rather closely, and pronounced influence of *Q. turbinella* is apparent in only a small minority.

TABLE 1. *Relation of collection sites to nearest meteorological observatory*

Observatory and elevation		Species and elevation		Distance
Ajo	1850 ft.	*Q. ajoensis*	2375–4000 ft.	35 mi.
Quartzite	900 ft.	*Q. ajoensis* × *Q. turbinella*	1800–3000 ft.	28–36 mi.
Seligman	5250 ft.	*Q. turbinella*	5250 ft.	0 mi.
Ashfork	5130 ft.	*Q. turbinella*	5130 ft.	0 mi.

distance and sometimes at higher elevations, but the low mountains in which the trees occur are insufficiently massive to induce strikingly lower temperatures. In contrast, two stations well within the range of typical *Q. turbinella* reveal quite a different climatological pattern. Seligman, in Yavapai County, with a 29 year record, at an elevation of 5250 feet, has an average annual precipitation of 11.31 inches, a January average temperature of 35.0° F., and a July average temperature of 72.2° F. Ashfork, in Yavapai County, with a 23 year record, at an elevation of 5130 feet, has an average annual precipitation of 13.3 inches, a January average temperature of 35.1° F., and a July average temperature of 74.1° F. (*op. cit.*). Thus, regardless of variations in precipitation, the Yavapai County localities, which are more or less characteristic of the woodland community of the plateau region where *Q. turbinella* is abundant, are characterized by low winter temperatures and moderate summer temperatures. The Pima and Yuma County localities, which are desert regions adjoining low mountains, where *Q. ajoensis* and its hybrids occur, are characterized by mild winter temperatures and high summer temperatures.

From these data it is clear that the present range of *Q. ajoensis* and its hybrids is not characterized by severe winters and that the woodland community of which *Q. turbinella* is a part is not characterized by severely hot summers. The confinement of *Q. turbinella* to woodland and of *Q. ajoensis* to waterways in the more xeric grassland would cast doubt upon drought as the factor responsible for the great discontinuity of the ranges of these species, although such a possibility cannot be eliminated. It is nearly impossible to separate high temperature effects from drought effects, but in this case the presence of ample moisture in the canyons of the Ajo Mountains would appear to be conclusive enough.

MORPHOLOGY

The Distinction of *Quercus ajoensis* from *Q. turbinella*

Herbarium specimens of these species show similarities to one another on several points: notably in the relatively small leaves, in their spinose marginal teeth and their glaucous gray-green coloration, and in the shape and size of the fruit—both the cup and the nut. A detailed analysis, however, reveals a number of morphological characters in which they differ. Indeed, they are actually so different that it was considered advisable to refer them to different taxonomic series within the subgenus *Lepidobalanus*.

The intermediate populations of Yuma County, and a few individuals of the Ajo Mountains population provide a source of possible confusion, since, obviously, they serve to "break down" the distinctions between the species. It is our conviction, however, that the intermediacy of the Yuma County populations is due to hybridization. Similarly, the aberrant individuals of the Ajo Mountains, we believe, provide evidence of introgression of that population by *Q. turbinella*.

Exclusive of these intermediates, there exist extensive populations of the two species between which sure distinction can readily be made. Specifically, we refer to (1) the greater portion of the Ajo

Mountains population of *Q. ajoensis,* occurring between 2800 and 4000 feet elevation, and (2) the more northerly and easterly portion of the broad range of *Q. turbinella,* in which there is no morphological evidence of genic infiltration of *Q. ajoensis.*

Considering, then, only relatively "pure" populations of the two species, they are distinguished by the differences listed in table 2.

The strikingly developed epidermal papillae of *Q. ajoensis* appear to be quite unusual, although not unique to this species. Schott (1900) in a study of leaf anatomy of 60 oaks of both the new and old world, stated that in the white oaks and *Q. chrysolepis* Liebm. the outer cell walls of the lower leaf epidermis are often thickened and strongly papillose to conical (p. 11). *Quercus chrysolepis* is the only specific example cited, however, and it is described elsewhere (p. 49) as being mostly weakly papillose. Metcalfe and Chalk (1950) stated that in *Quercus* the lower leaf epidermis sometimes tends to be papillose. These authors cite a single reference, the work of Holm (1910), who reported that in *Q. alba* L. "near the stomata the epidermis forms low papillae."

We have examined characteristic specimens of *Q. chrysolepis* and *Q. alba,* as well as a number of species related to *Q. ajoensis.* Suffice it to say that the development of papillae characteristic of *Q. ajoensis* (cf. fig. 2, A) greatly exceeds—both in abundance and degree of development—anything seen in the two previously named species. Indeed, in the white oaks we have examined thus far, only *Q. hinckleyi* C. H. Mull. and *Q. perpallida* Trel. exhibt papillae approaching at all closely in degree of development those of *Q. ajoensis. Q. hinckleyi* is a close relative of *Q. ajoensis,* forming, with

TABLE 2. *Morphological differences between* Q. ajoensis *and* Q. turbinella

	Q. ajoensis	*Q. turbinella*
Lower leaf surface pubescence	glabrous	moderately to densely stellate-pubescent and distinctly glandular-puberulent
epidermal papillae	abundant and strongly developed	lacking
color	very glaucous	often markedly golden (from the abundant yellow glandular puberulence)
Leaf margin	marginal spine-tipped teeth very long and attenuated, commonly up to 6 mm. long on moderate-sized leaves (extreme teeth on large leaves 8 or more mm. long)	marginal teeth less strikingly developed, up to 5 mm. (or commonly less) in length
Leaf size		
length	22–38 mm. (or more), commonly ca. 30 mm.	16–28 mm. (or more), commonly ca. 23 mm.
width	11–25 mm. (or more), commonly ca. 17 mm.	11–17 mm. (or more), commonly ca. 13 mm.
Young emerging foliage	moderately to strongly anthocyanous	very weakly or (usually) not at all anthocyanous
Twig pubescence	glabrous	densely stellate-pubescent

FIG. 1. Southwestern United States showing the distribution of *Quercus ajoensis*, *Q. turbinella*, and hybrid populations.

it, the series *Relictae;* and *Q. perpallida* is a member of the closely related series, *Glaucoideae.*

In addition to the differences listed in table 2, the acorn cups exhibit several differences, which, however, appear to be less constant than those in the table. In *Q. ajoensis* the apex of the cup-scale is typically short ligulate to subligulate, glabrous, and chestnut brown in color. This color of the tips contrasts sharply with the light buff of the basal portions of the scales, imparting a striking checkered appearance to the cup as a whole (see Muller, *op. cit.*, fig. 1). In *Q. turbinella,* the apex of the cup-scale is commonly nonligulate (although this character is variable), puberulent, and only slightly (if at all) darker in color than the light buff of the basal portion of the scale.

In growth habit the typical subspecies of *Q. turbinella* is commonly a shrub, or infrequently a small shrubby tree up to 15 feet in height. *Quercus ajoensis,* as it occurs along the stream bed of Alamo Canyon, is a shrubby tree up to 30 feet in height. The larger individuals have trunks to as much as 2 feet in basal diameter. On open, north-facing slopes at the head of Alamo Canyon, however, where it is certainly no less pure genetically, it occurs as a shrub to 6 feet in height.

Population Samples

Methods

In order to obtain a clear picture of the pattern of variation in the populations of the Ajo, Castle Dome, and Kofa Mountains, these were sampled extensively and systematically by the authors. For the purpose of making comparisons with *Q. turbinella,* a population sample of this species from the New York Mountains, of eastern San Bernardino County, California, was used. The locations, number of individuals, etc., for these popula-

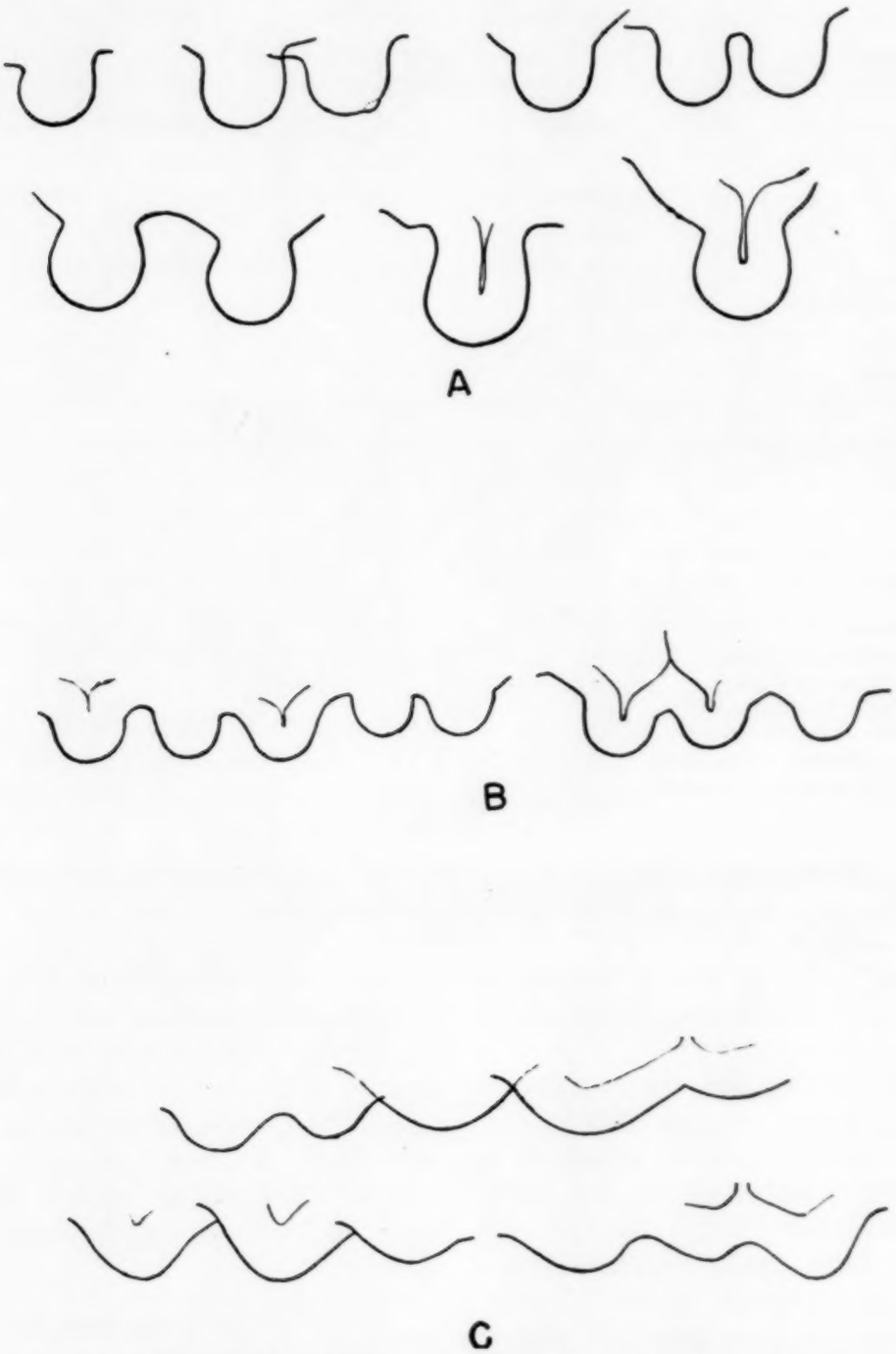

FIG. 2. Degrees of development of epidermal papillae as seen in transverse section: A. strongly developed, B. moderately developed, C. slightly developed. (All figures × ca. 430.)

tion samples are given in table 3. Complete duplicate sets of all the specimens involved in the analyses are preserved in the herbarium of the University of California at Davis and in the private herbarium of C. H. Muller at Santa Barbara.

These collections were analyzed with regard to five of the specific differences listed above: (1) relative abundance of glandular puberulence, and (2) of stellate trichomes, (3) degree of development of epidermal papillae, (4) leaf size, and (5) length of marginal teeth.

Glandular puberulence. For determining the relative abundance of glandular puberulence, the lower surfaces of 10 mature, representative leaves were examined under 30 × magnification and each was

scored as follows. Four categories were arbitrarily recognized: (a) absent or extremely sparse (score: 0), (b) sparse (1), (c) moderate (2), and (d) abundant (3). The sum of the 10 values was taken as an individual's score on this character. A "standard" leaf for each of the four conditions was kept at hand for comparison during the analysis.

The relative abundance of these glandular trichomes is often a function of age. They are frequently present in greater abundance in young (but fully expanded) leaves than in older, fully matured leaves. For the analysis of this character difference, young leaves would probably have given a better measure of the genetic potential *to produce* glandular trichomes (since the relative degree of deciduousness of the trichomes may be controlled by other independent factors). Since some specimens lacked young leaves, however, this was out of the question. Therefore, young as well as old, weatherbeaten leaves were avoided.

Stellate trichomes. For determining the relative abundance of stellate trichomes, an ocular grid was used which encompassed an area of approximately one square mm. at 100 × magnification. A single count (the number of stellate trichomes within the area of the grid) was made from the lower surface of each of 10 representative leaves for each specimen. The mean of these 10 values was taken as an individual's score on this character. The relative abundance of these trichomes, also, seems to be a function of

TABLE 3. *Population samples of* Quercus ajoensis, Q. turbinella, *and hybrid populations*

Taxonomic classification	Collector and number	No. of individuals in sample	Date	Location
Q. ajoensis	Tucker and Muller 2426	36	Aug. 15, 1952	Lower Alamo Canyon,* Ajo Mtns., Pima Co., Arizona
Q. ajoensis	Muller and Tucker 9535–9542 and 9544–9546	11	Apr. 27, 1953	Four locations in upper Alamo Canyon, Ajo Mtns.; at elevations of 2800 ft., 3200 ft., 3500 ft., and 4000 ft.
Q. ajoensis	Muller and Tucker 9547–9550	4	Apr. 27, 1953	Two locations in Arch Canyon, Ajo Mtns.; at elevations of 3200 ft. and 2500 ft.
Hybrid population	Muller 9526–9531	6	Aug. 16, 1952	Canyon above Horse Tanks, Castle Dome Mtns., Yuma Co., Arizona; elevation about 1800 ft.
Hybrid population	Muller 9532–9534	3	Aug. 16, 1952	Palm Canyon,** Kofa Mtns., Yuma Co., Arizona; elevation about 2500 ft.
Hybrid population	Tucker 2616	11	Sept. 5, 1952	Palm Canyon, Kofa Mtns.; elevation about 2500 ft.
Hybrid population	Tucker and Muller 2641–2642	15	Apr. 29, 1953	Two locations in Palm Canyon, Kofa Mtns.; head of Palm Canyon, and middle Palm Canyon.
Q. turbinella	Tucker 1875	26	Sept. 25, 1948	New York Mtns., ca. 8 mi. s. of Ivanpah, San Bernardino Co., California

* For purposes of presentation and comparison of data (cf. figs. 4–12), all these collections from Alamo and Arch Canyons, Ajo Mtns., were treated as a single composite population sample.

** All collections from Palm Canyon, Kofa Mtns., were treated as a single composite population sample.

age. Hence, old, weatherbeaten leaves were avoided.

Epidermal papillae. For studying this character, two representative leaves were selected from each specimen, and a slide made for each of them, following this procedure: they were first softened by boiling for about ½ hour in distilled water with a small amount of detergent added, and then transferred to 50% ethyl alcohol. Leaf segments devoid of the midrib and other major veins were cut out. From these, free-hand transverse sections were cut, and about 14–16 of the thinnest mounted in lactophenol. These slides were made semi-permanent by ringing the cover slip with one of several sealing compounds—"Cutex" nail polish, "Gold Size," and others. They are all on file in the Botany Department of the University of California, Davis.

It soon became apparent that scoring this character would not be easy, for it was not merely a matter of observing presence or absence of papillae, but of evaluating their degree of development as well. On the latter point, three arbitrary categories were recognized: (1) strongly developed, (2) moderately developed, and (3) absent to slightly developed (see fig. 2). Moreover, considerable variation was sometimes encountered even in different portions of a single section. An attempt was made, therefore, to develop a method of scoring that would not ignore such variation, and yet would render a single score for the individual. Each of ten sections per slide was scored using the following scale: absent to slight (score: 0); mostly slight, to moderate (1); slight to moderate (2); slight to mostly moderate (3); moderate (4); mostly moderate, to strong (5); moderate to strong (6); moderate to mostly strong (7); and strong (8). The two slides for each specimen were thus scored, the 20 resulting values were averaged, and this mean value was taken as the individual's index score.

The typical condition of this character in *Q. turbinella* over its range as a whole was determined by similarly analyzing 31 miscellaneous herbarium specimens—21 of *Q. turbinella* subsp. *turbinella* from widely scattered parts of its range from California to Texas, and 10 of *Q. turbinella* subsp. *californica* Tucker, which is restricted to California.

Leaf size. The length and width (to the nearest mm.) of 10 representative leaves per specimen were tabulated. Length, alone, however, was taken as a satisfactory index of leaf size, for in general there was an obvious positive correlation between length and width, and little if any difference between these populations in the length: width ratio. The mean of the 10 length measurements was taken as an individual's index score.

Length of marginal teeth. One of the most conspicuous differences between *Q. ajoensis* and *Q. turbinella* is in the greater development of the teeth along the leaf margin in the former. However, since

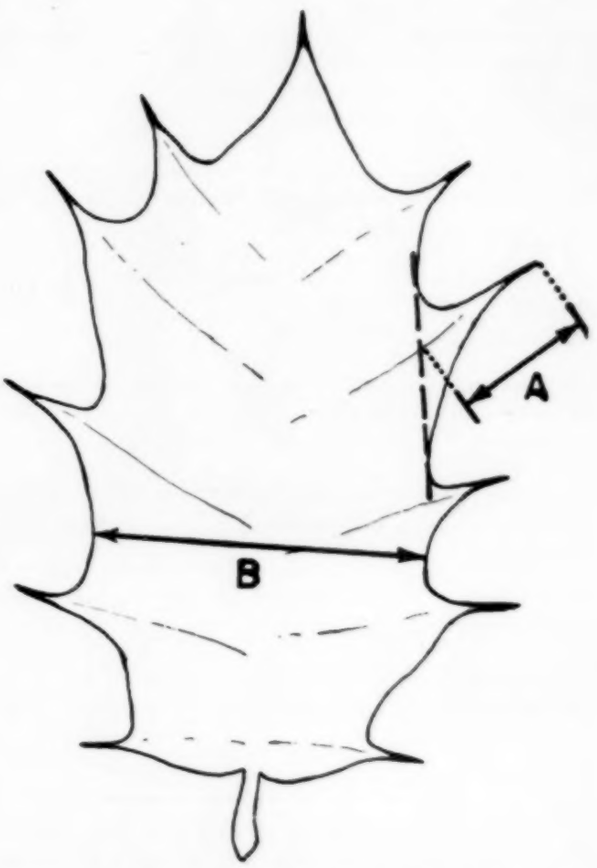

FIG. 3. The measurements taken for tooth length (A), and leaf width (B).

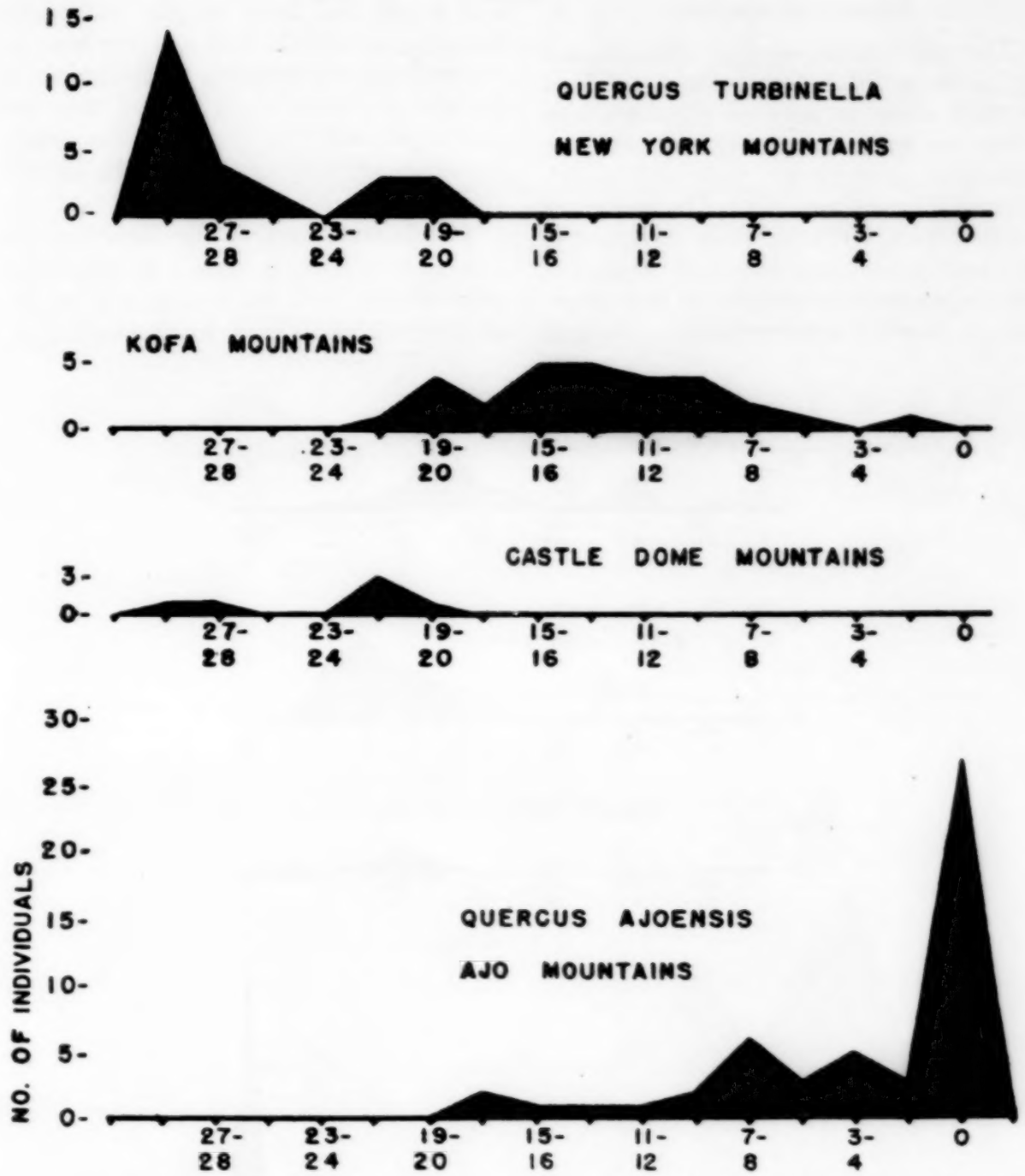

FIG. 4. Frequency distributions of the relative abundance of glandular puberulence. (Horizontal scale represents the range of index scores; see explanation in text.)

Q. ajoensis also has larger leaves on the average, and since tooth length and leaf size are probably positively correlated, in order to express tooth length as an independent character, the ratio of tooth length to leaf width was used instead.

The following procedure was used: ten representative leaves per specimen were measured—extremes being carefully avoided. Using a dissecting microscope with 10 × magnification and an ocular micrometer, the length of a single lateral tooth—usually the most strongly developed—and the maximum width of the middle portion of the lamina, exclusive of the teeth (see fig. 3), were recorded. The ratio of the former value to the latter was computed, and the mean of the 10 values thus obtained for each specimen was taken as its index score.

Results of Analysis

The populations of *Q. turbinella* and *Q. ajoensis* differ considerably (at least in their mean values) on all characters. They are most completely separated on glandular puberulence and stellate trichomes (figs. 4 and 5). On epidermal papillae (fig. 6), also, most members of the two populations are well separated, although a small proportion of both populations overlap conspicuously. In both leaf length and tooth length: leaf width ratio (figs. 7 and 8), however, the amount of overlap is considerable (although when population means are computed, they are seen to be widely divergent). Our interpretation of this overlapping in certain characters is presented below.

The intermediacy of the Kofa population is most clearly shown by glandular puberulence, and somewhat less so by stellate trichomes and tooth length: leaf

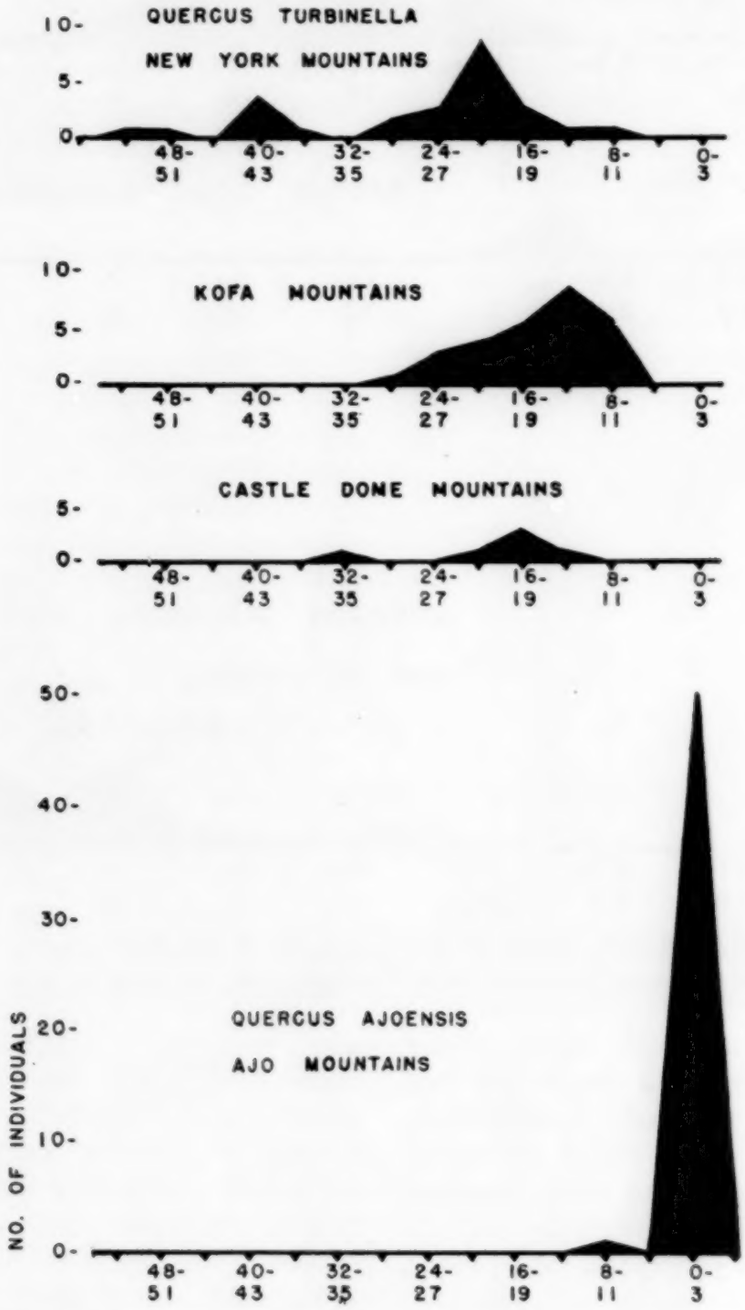

FIG. 5. Frequency distributions of the relative abundance of stellate trichomes.

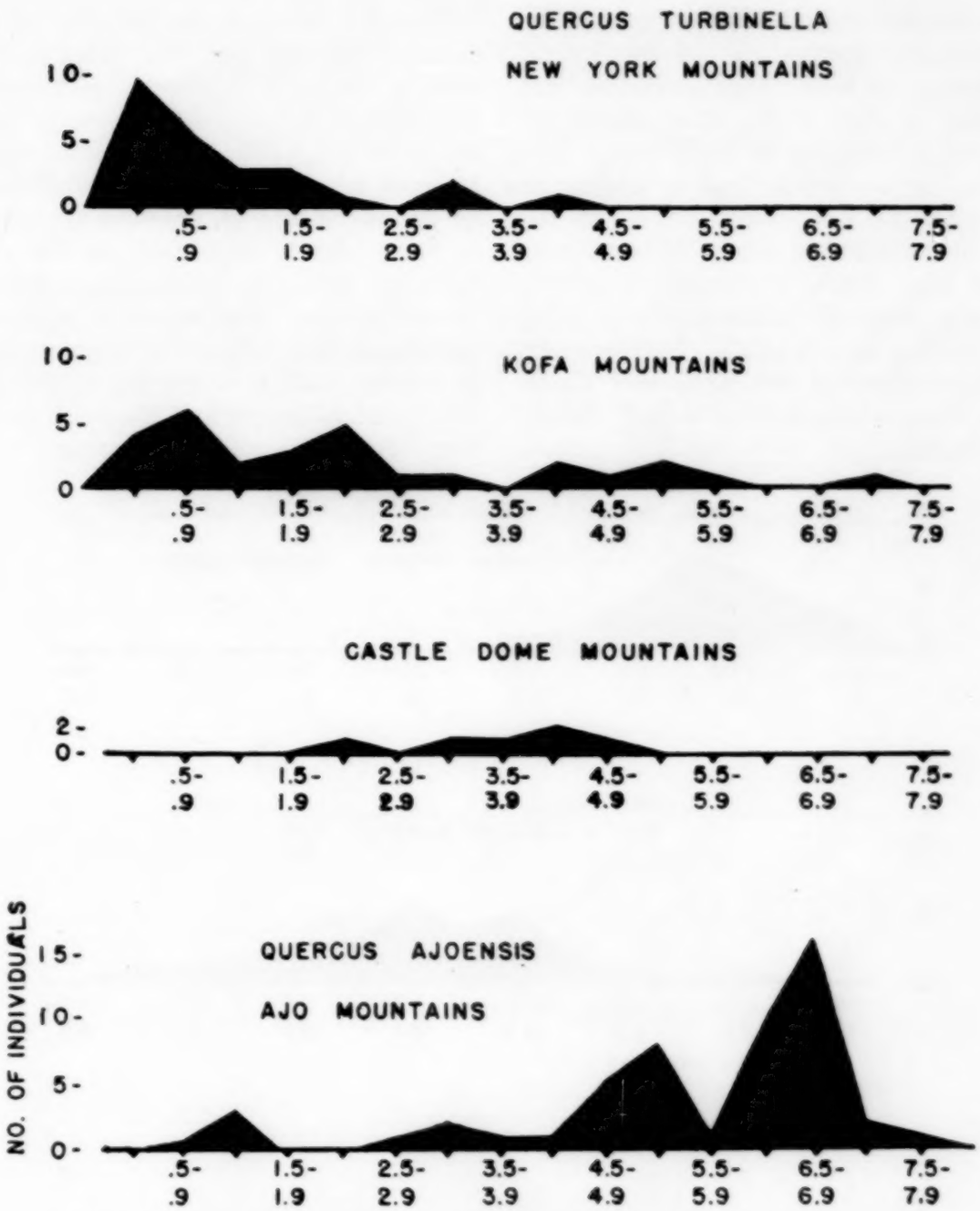

FIG. 6. Frequency distributions of the degree of development of epidermal papillae.

width ratio. The Castle Dome population is most clearly intermediate in epidermal papillae. However, the mean of this population on every character except the tooth length: leaf width ratio falls between those of the New York Mountains and the Ajo Mountains.

The distinctness of *Q. turbinella* and *Q. ajoensis,* however, as well as the intermediacy of the populations of the Kofa and Castle Dome Ranges, is best shown graphically by an aggregate character index. Such an index, similar to that developed by Hubbs, Hubbs, and Johnson (1943, p. 7), based on the scores of all five characters, is shown in figure 9. Considering each character in turn, the mean value for the population sample of the parental species having the lower values was set at 0. The mean value for the other parental species was set at 100. On a proportional basis, values were then

computed for all individuals—both of the two parental species and of the hybrid populations, as well. This procedure was repeated for each of the other characters analyzed, and aggregate index totals were obtained for each individual by adding its scores for all five characters. On the basis of such index totals, *Q. turbinella* of the New York Mountains is clearly separable from *Q. ajoensis,* there being no overlap of scores at all. Furthermore, the populations of the Kofa and Castle Dome Ranges are, at least in part, clearly intermediate.

In order to show the patterns of correlation between the five index characters in these populations, pictorialized scatter diagrams similar to those proposed by Anderson (1949) are used in figures 11 and 12. Again, the populations of *Q. turbinella* and *Q. ajoensis* are seen to be most clearly separated on the characters of glandular puberulence and stellate trichomes. The scores on epidermal papillae show a rather remarkable amount of overlap, and, as noted above, the characters of leaf length and the tooth length: leaf width ratio show even more. This

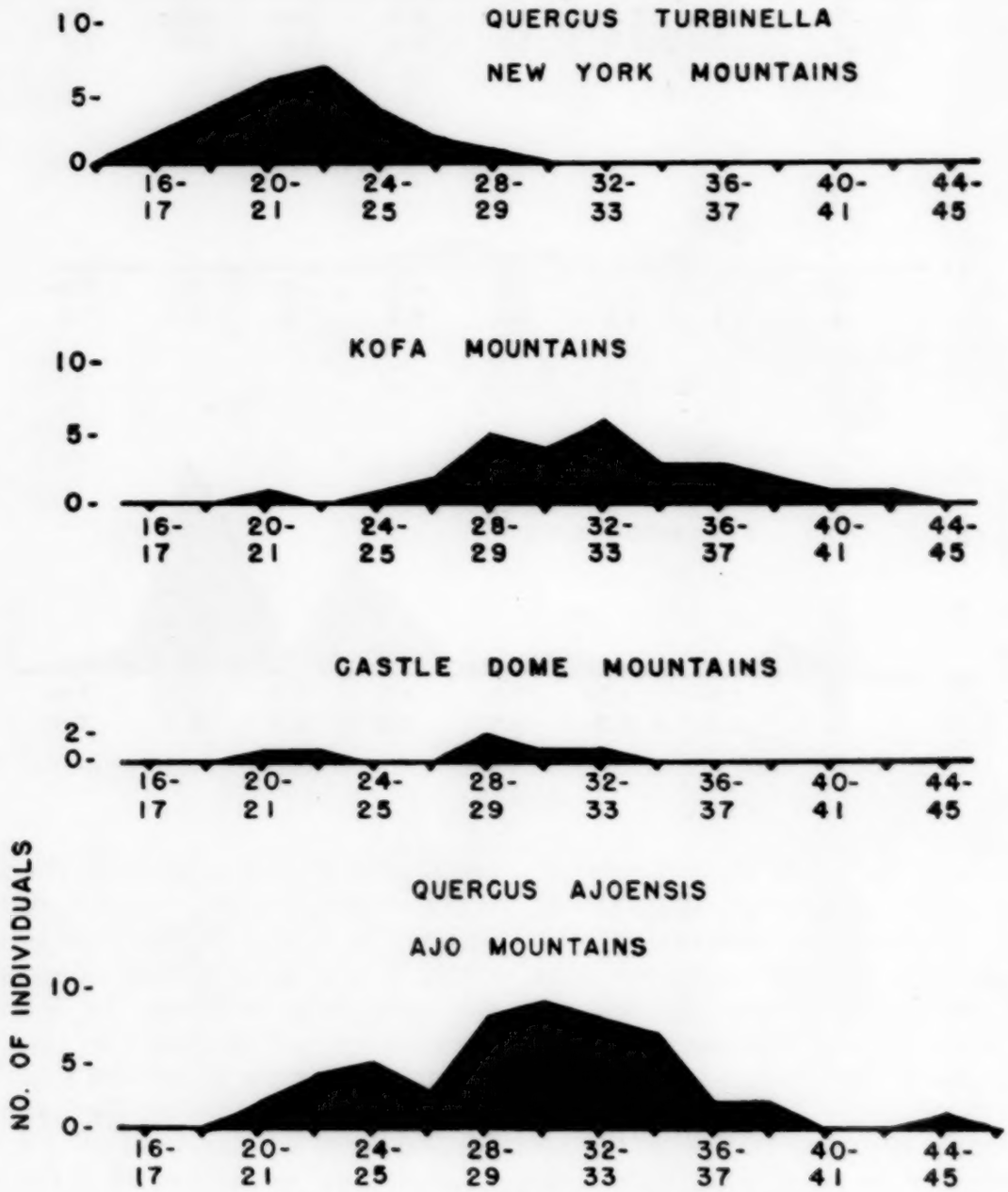

FIG. 7. Frequency distributions of leaf length.

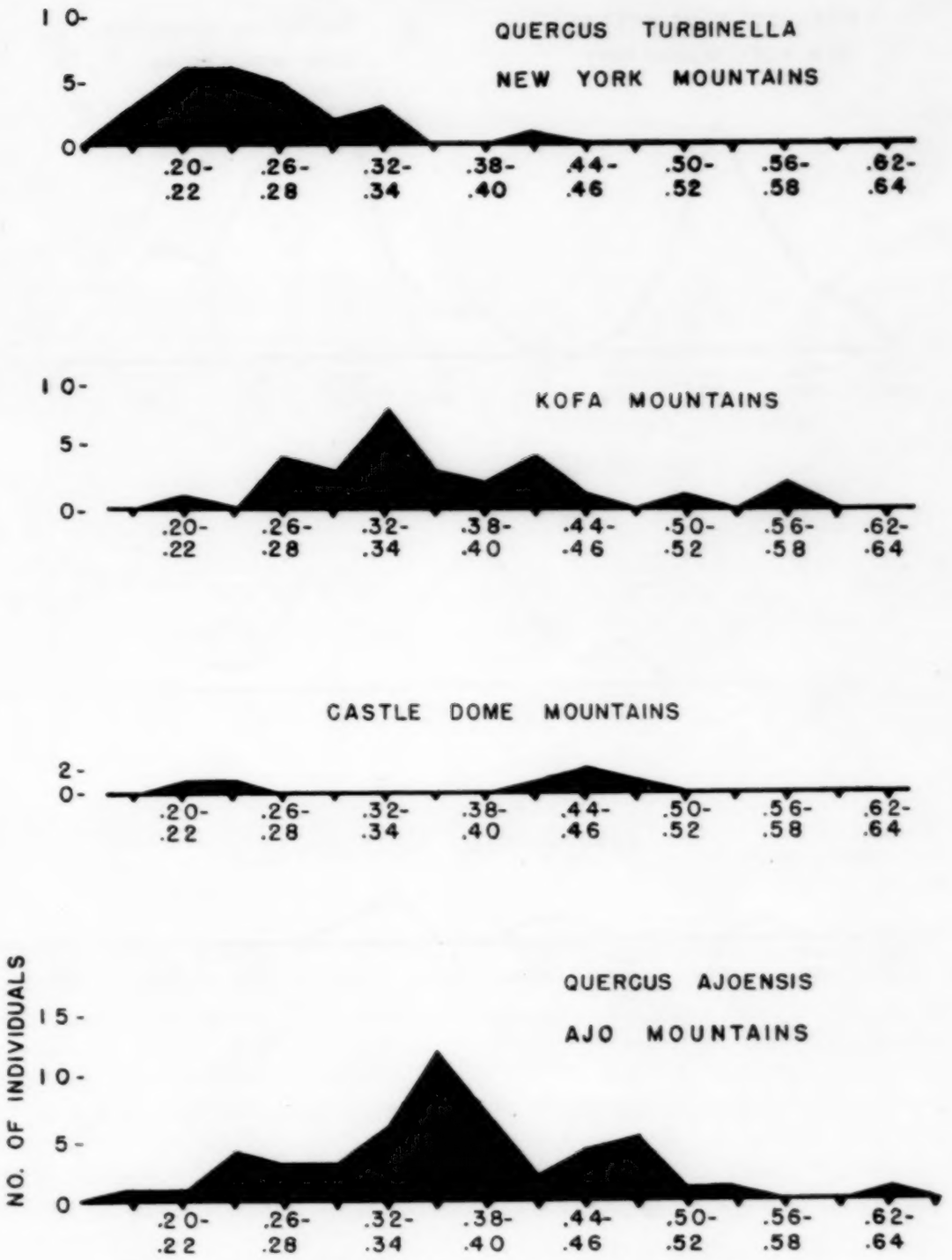

FIG. 8. Frequency distributions of tooth length: leaf width ratios.

large amount of overlap in either of the two last-named characters undoubtedly is partly due to the fact that the normal range of variation of one species overlaps that of the other. Therefore, although the "intermediate" range in these characters is designated by special symbols for the purpose of our pictorialized scatter diagrams, these should be interpreted with reservations. In any population it is clearly impossible to determine from these data whether an individual with "intermediate" leaf length, for example, is merely an extreme (but genetically "pure") individual of its species, or is actually a hybrid derivative, and intermediate by virtue of genetic heterozygosity.

We interpret the overlap in index

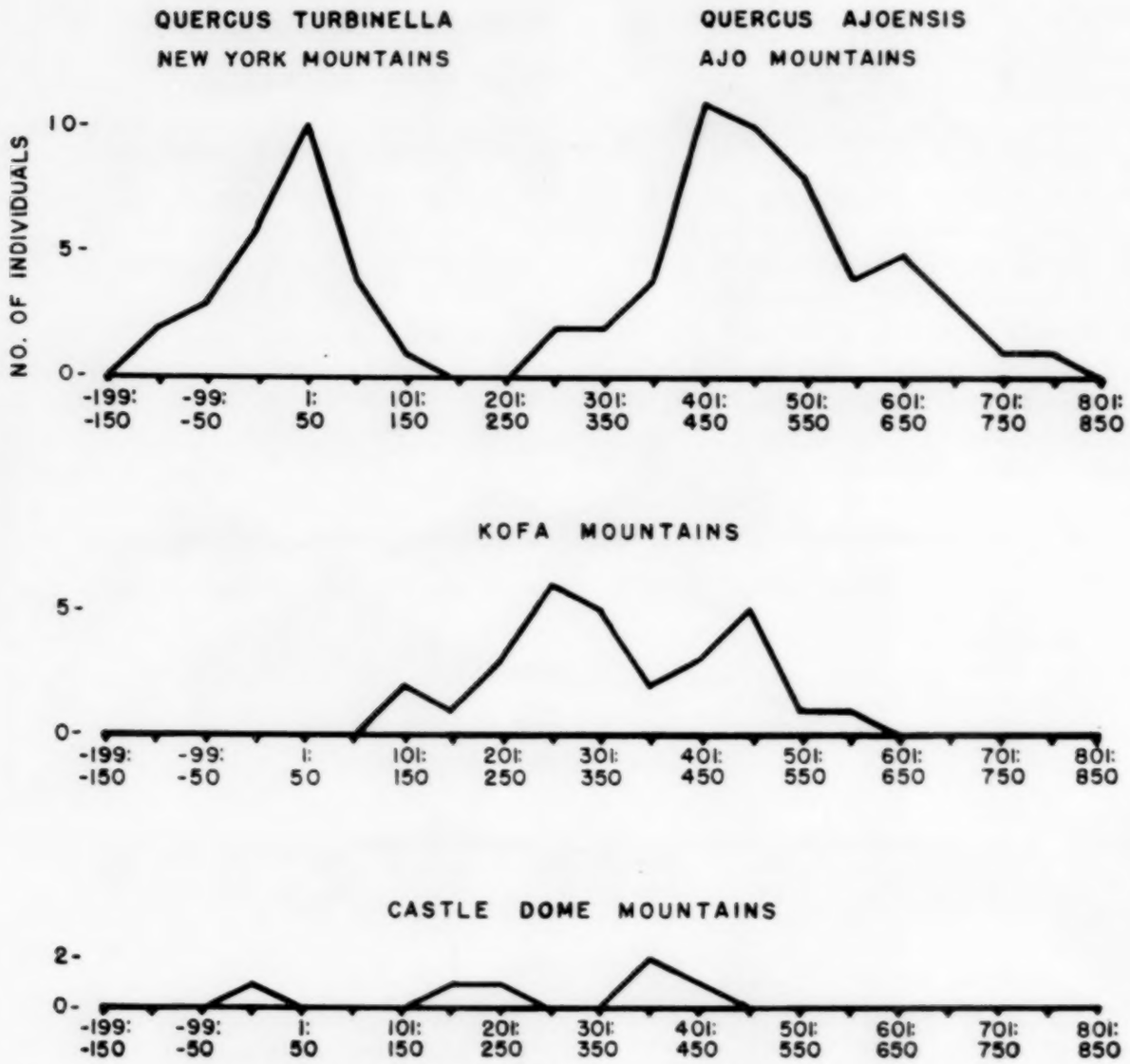

FIG. 9. Aggregate index based on all five characters (see explanation in text).

	Q. turbinella	Intermediate	Q. ajoensis
Stellate Trichomes	● (score: over 16)	⊙ (score: 1.1-16.0)	○ (score: 0.0-1.0)
Tooth Length: Leaf Width Ratio	○ (up to & incl. .28)	○ (.29-.33)	○ (.34 and over)
Leaf Length	○ (up to & incl. 23)	♂ (24-27)	♂ (28 and over)

FIG. 10. Explanation of symbols used in pictorialized scatter diagrams.

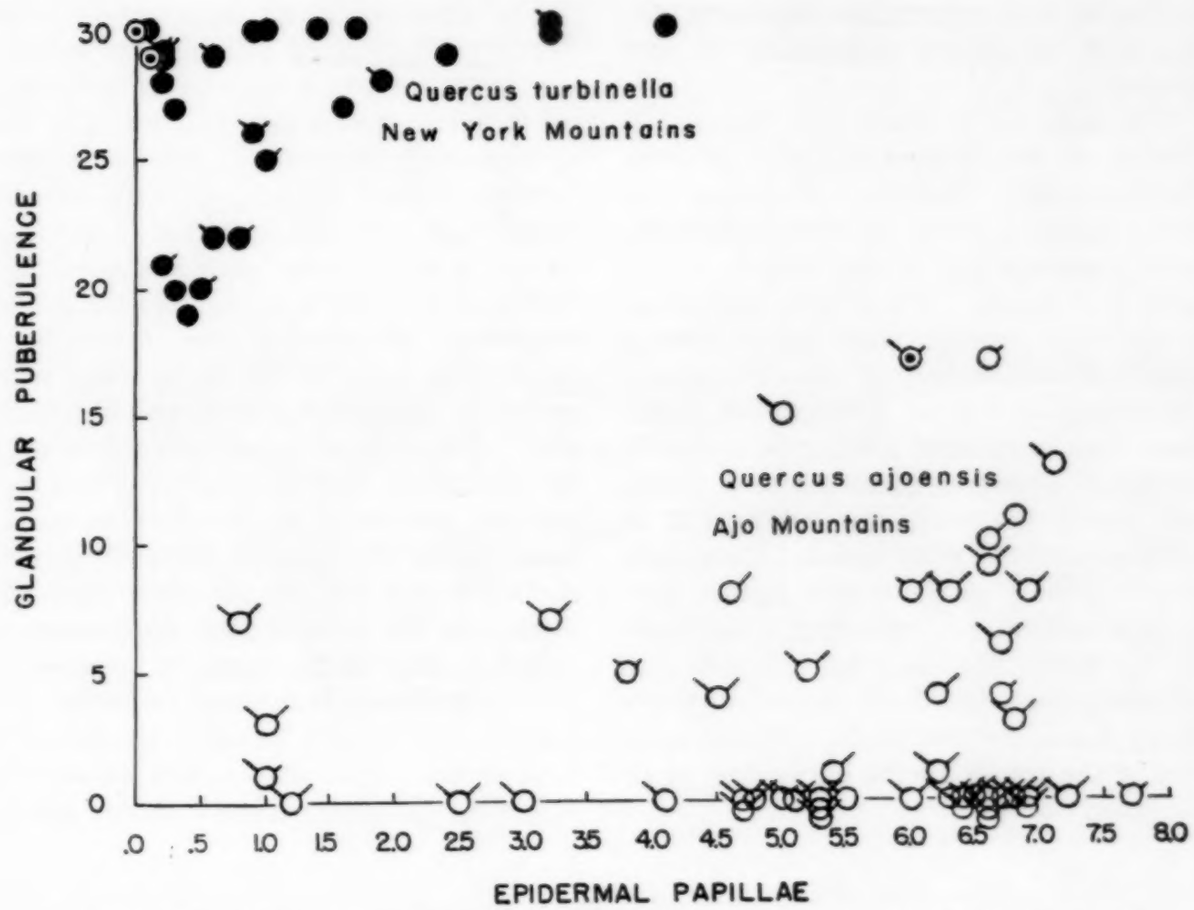

FIG. 11. Pictorialized scatter diagram showing the correlation between all five index characters in population samples of *Q. turbinella* and *Q. ajoensis* (see fig. 10 for explanation of symbols).

scores for epidermal papillae, however, as being due in large part to reciprocal introgression. In the New York Mountains population of *Q. turbinella* the appreciable index scores in a few individuals seems almost certainly to be the result of introgression. A number of miscellaneous herbarium specimens from the same general portion of the range of the species also show some degree of papillar development (the special significance of this will be explained later), but specimens from regions most remote from *Q. ajoensis* consistently have index scores of 0 (fig. 13). Conversely, in the population of *Q. ajoensis,* the very low scores shown by a few individuals are just as logically interpreted as being due to the influence of *Q. turbinella* genes as are the traces of stellate pubescence and especially glandular puberulence to be seen in a few individuals.

The Kofa and Castle Dome populations (fig. 12) are, in general, intermediate, although the latter is somewhat closer to *Q. turbinella.* The Kofa population, on the other hand, is more nearly intermediate except in leaf length and in the tooth length : leaf width ratio—in which it resembles *Q. ajoensis* more closely. (This is more readily apparent in figs. 7 and 8.)

PALEONTOLOGICAL EVIDENCE

The fossil record is strikingly poor in reference to our problem except for one wholly unexpected find. A careful study of the microphyllous fossil floras of Late Tertiary age in California and adjacent Nevada revealed not a single leaf suggestive of *Q. ajoensis,* although a scattered few leaves of Pliocene age were discovered (from localities within the range of living *Q. turbinella* subsp. *californica*) which were clearly representative of *Q.*

turbinella and apparently represent the Arizonan or typical subspecies of that species.

Through the courtesy of Mr. C. J. Smiley of the University of California Paleobotanical Museum, Berkeley, we were shown a series of three extremely well preserved leaves that clearly represent *Q. ajoensis*. The fossils, named *Q. ajoensoides* Smiley, were taken from a Lower Pliocene bed in the Ellensburg Formation in central Washington where they were associated with a wide variety of woody genera a great many of which are warm temperate to subtropical in their present day distribution. Extremely close checking of the fossils against herbarium specimens (including a duplicate of the type collection) by botanists and paleobotanists failed to reveal a single detail that could cast doubt upon the identity of the fossils as the equivalent of *Q. ajoensis*.

The presence of *Q. ajoensis* in Late Tertiary deposits of Washington associated with a warm temperate or subtropical flora has certain significances: (1) the species once occurred in the far northwestern United States; (2) its present range represents a contraction of the fossil range that agrees with the northerly distribution of the hybrids; (3) the warm temperate affinities of the fossil flora agree with those of the living flora with which *Q. ajoensis* has been and is associated. From these considerations it may be concluded that *Q. ajoensis* was restricted southward as lowering temperatures forced the tropical elements out of the north and that for the same reason it occurs in its present day northernmost outposts only in the form of hybrids of *Q. turbinella*. It is unusual for living and Late Tertiary oaks to agree in such minute detail. This may reflect an ancient origin for *Q. ajoensis*, but it almost surely

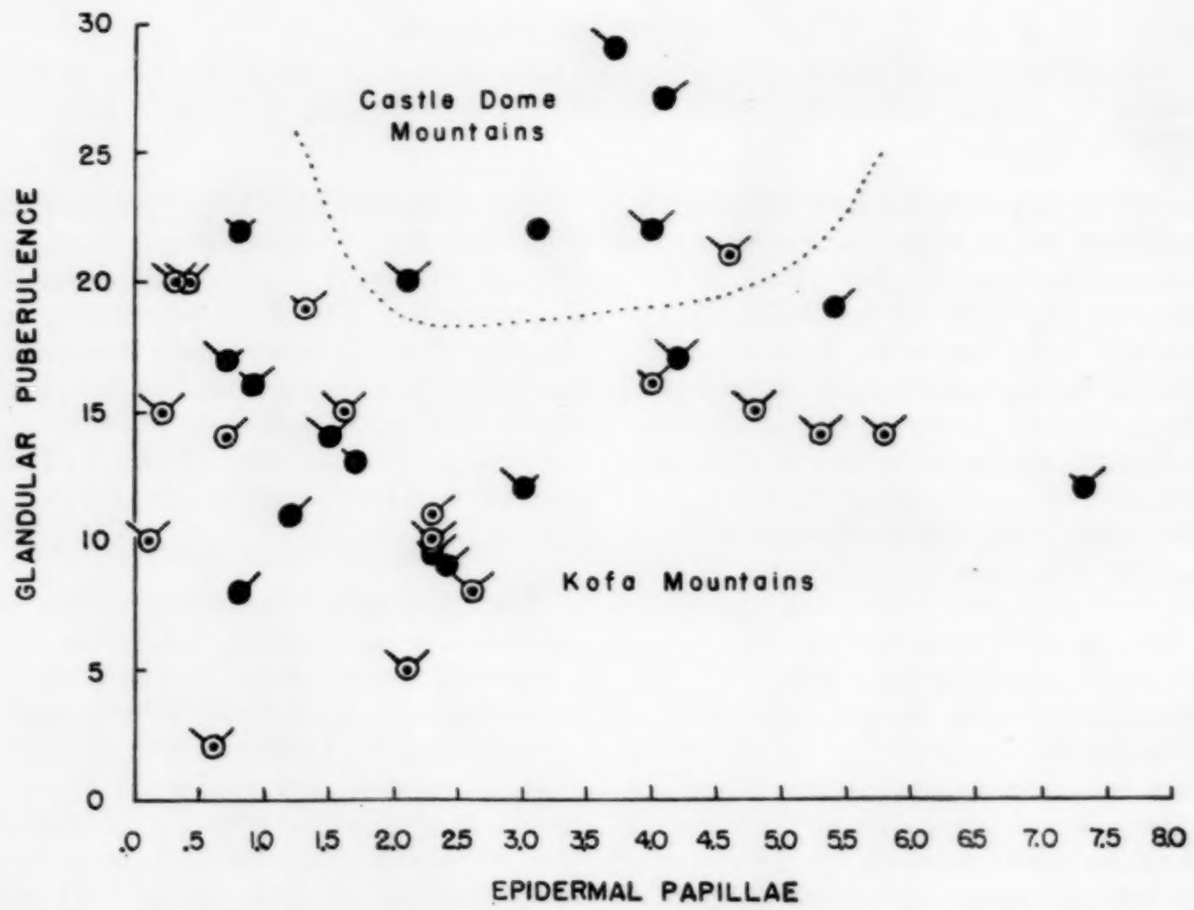

FIG. 12. Pictorialized scatter diagram showing the correlation between all five index characters in samples of the hybrid populations (see fig. 10 for explanation of symbols).

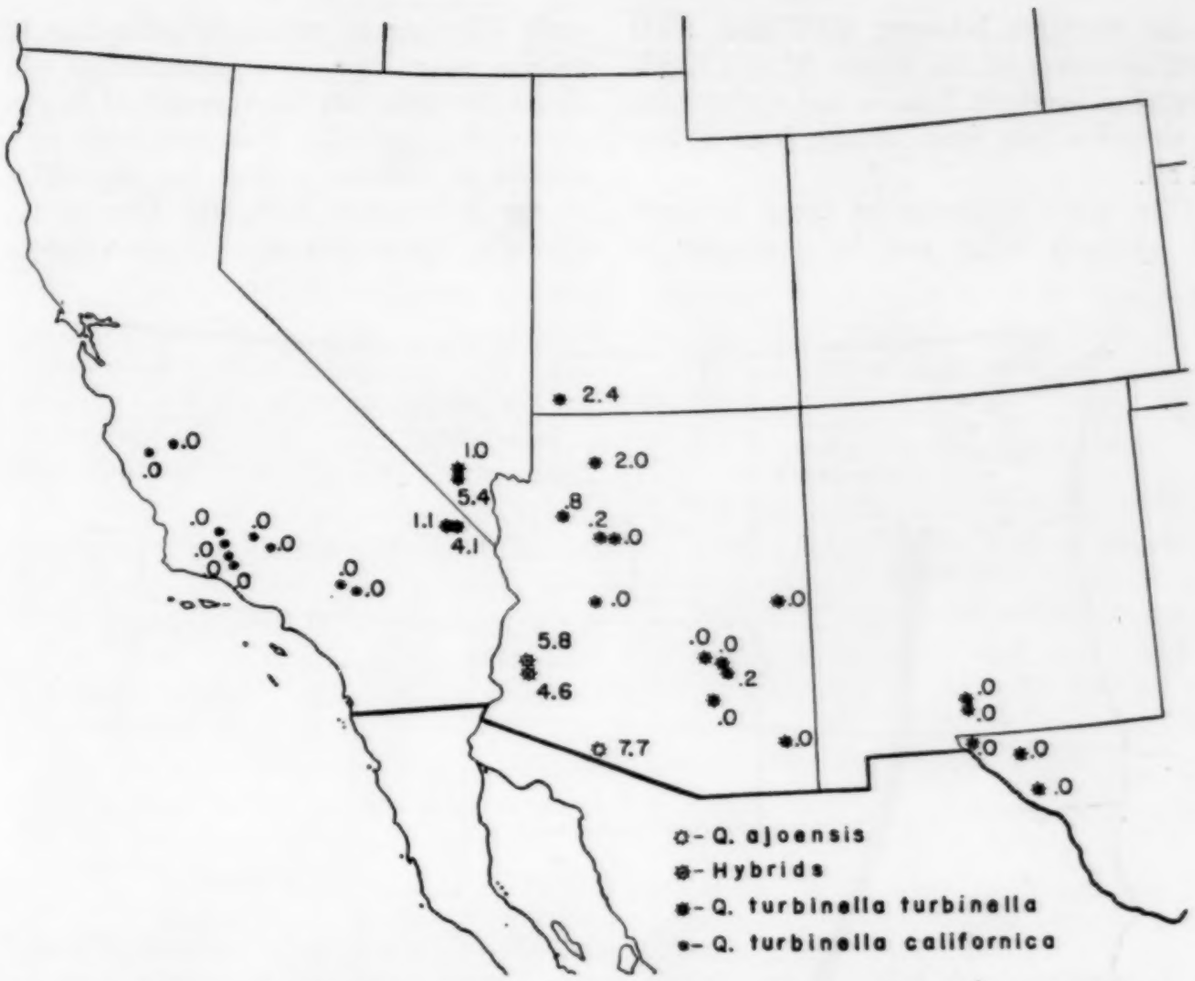

FIG. 13. Collections analyzed for foliar epidermal papillae. The symbol indicates the location and taxonomic classification. The accompanying number is the index value obtained.

indicates a lack of variability similar to the uniformity of the living population in the Ajo Mountains. To the extent that morphological uniformity and physiological uniformity are correlated, this may indicate an inherent poverty of biotypes on the basis of which the species is now drastically restricted in range. Widespread and uniform species are commonplace in the widespread and uniform tropical habitats, quite unlike the degree of variability of species and habitats of temperate regions, whether these result from increase in latitude or in altitude.

DISCUSSION

A number of facts indicate that *Q. ajoensis* is subtropical in nature. In addition to its subtropical habitat requirements, discussed previously, its most closely related species seem to be subtropical in nature, also. Its closest relative, with which it forms the series *Relictae,* is *Q. hinckleyi* C. H. Mull., of semi-desert West Texas. (The two species share the following characters: glaucous leaves, papillae on the lower surface, pronounced marginal spines, and prominent anthocyanous coloration of the young foliage.) The closest relatives of the *Relictae* are the species forming the Mexican series *Glaucoideae,* to which it is similar in a number of respects, but from which it differs in the presence of papillae and the prominent marginal spines. Interestingly enough, one species in the *Glaucoideae—Q. perpallida* Trel.—*does* exhibit papillae on the lower leaf surface. This species, closest of the *Glaucoideae* to *Q. ajoensis* morphologically, likewise seems confined to subtropical habitats. It is concentrated in

moist canyons between 4000 and 5000 feet elevation in the Sierra Madre Occidental of southern Sonora and Chihuahua, a situation free from severe frost if any at all.

The great disparity in range between *Q. ajoensis* today and its precursor in early Pliocene of central Washington 10 million years ago is a reflection of climatic changes that have occurred in the intervening period. This may have proceeded as follows. After the deposition of the Ellensburg flora, the rise of the Cascade Mountains and Sierra Nevada

FIG. 14. Western United States showing the present distribution of *Q. ajoensis* (A), and the Lower Pliocene (Ellensburg) occurrence of its Tertiary equivalent, *Q. ajoensoides*. The arrow indicates its migration (or the contraction of its range) southward in post-Lower Pliocene time. (Extent of present range of *Q. turbinella* enclosed by hatched lines; portions of its range in which epidermal papillae occur indicated by stippling; location of hybrid populations: B.).

resulted in greater continentality of the climate to the east of these ranges (Chaney, 1936, pp. 75–76). The warm temperate flora of that time and region was excluded by lowering temperature and rainfall (Chaney, 1944, pp. 353–354) so that *Ulmus, Juglans, Liquidambar, Zelkova, Paulownia, Umbellularia, Quercus winstanleyi* Chaney, and *Q. ajoensoides* Smiley were all eliminated. If *Q. ajoensis,* through its Pliocene equivalent, *Q. ajoensoides,* had a wide distribution at that time, its elimination from the northern end of its range by low minimum temperatures under continental conditions and its elimination from the southern lowlands by progressively increasing drought would have resulted in its present confinement to moist situations in a desert area of mild winters.

We suggest, therefore, that *Q. ajoensis* (or some part of it) has several times become relictual. *First,* it persisted in central Washington after the subtropical early Tertiary flora of that area was eliminated, and came to represent (by Ellensburg time or early Pliocene) the last subtropical species in the flora. *Second,* it was eliminated progressively from the north and persisted southward. Thus, if one examines the present population of *Q. turbinella* for the papillae of *Q. ajoensis,* specimens of the subspecies *californica* are found to be free of papillae; so, also, are specimens of the typical subspecies from western Texas, southern New Mexico, and southeastern Arizona. However, definite development of papillae in varying degree are to be found in some collections of the latter subspecies from northwestern Arizona, southwestern Utah, southern Nevada, and adjacent southeastern California (fig. 13). In other words, the influence of the genes of *Q. ajoensis* (as regards papillae) is recognizable in that tongue of the population of *Q. turbinella* that lies across the migration route of *Q. ajoensis* between central Washington and southwestern Arizona. *Third,* in the Kofa, Castle Dome and Eagle Tail Mountains of Yuma County, Arizona, there occur intermediate populations that represent a series of early hybridizations from which *Q. ajoensis* has persisted only in the form of relictual hybrids. *Fourth* and finally, *Q. ajoensis* has persisted as a relic in the moist localities of the Ajo Mountains (fig. 14) because here it is subject neither to the drought of the surrounding desert nor to the low winter temperatures of its previous northerly range.

Acknowledgments

We are indebted to Professor Herbert L. Mason for critically reading the manuscript. Drs. Kittie Parker and Charles T. Mason, Jr. kindly furnished collections, and data from herbarium specimens at the University of Arizona Herbarium. Miss June McCaskill assisted with the preparation of the illustrations.

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COMPARISON OF THE BLOCK ISLAND "SPECIES" OF *MICROTUS* WITH *M. PENNSYLVANICUS*

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INTRODUCTION

Block Island is a small continental island, about 11 square miles in area, located approximately 15 miles northeast of Montauk Point, L. I., N. Y., and southwest by south of Point Judith, R. I. (see figure 1). Several years ago it was suggested that the Microtus inhabiting the island were sufficiently different morphologically from the field mice on the neighboring mainland (*Microtus pennsylvanicus*) to warrant species status for the island mice (*Microtus provectus*) (Bangs, 1908). Recent work has shown that the two populations are not sexually isolated to a degree which prevents the production of F_1 hybrids (Wheeler, 1952) and in view of these findings, it seemed essential to reexamine Microtus from both localities not only to confirm and more thoroughly analyze the differences already indicated by Bangs (1908) but to determine whether other differences do exist which would indicate genetic divergence to any degree.

The insular species, *Microtus provectus,* has been described, first of all, as somewhat larger than the mainland Microtus. In view of this, the present investigation includes a statistical analysis of the body size of mice from both insular and mainland populations in addition to a comparison of body lengths of mature specimens within each population. Since other size differences associated with the skull were also suggested (Bangs, 1908), the experimental data in this paper include size comparisons of some of the individual skull bones and comparisons of various measurements made on the skulls as a whole.

MATERIAL AND METHODS

All mice which were available for this study were caught by standard snap-type mouse traps placed within the runways of the field mice. It did not prove advantageous to use bait and therefore time was saved by eliminating this procedure soon after the work was begun.

Although the majority of the mainland Microtus was caught in the vicinity of New London, some mice from the mainland were trapped near New Haven (see figure 1). All mainland Microtus were taken during a trapping period which extended from February 1949–March 1950. The insular Microtus were caught on Block Island during the summer of 1949 between July 31 and August 4.

The lengths of the bodies, tails, feet, and ears of the mice were recorded with a millimeter ruler as soon as was possible after removing the animals from the traps. Following this, study skins were prepared and the skulls were cleaned in preparation for future study.

Some dimensions of the skulls as a whole were measured directly from the skulls with vernier calipers. In addition, the area of the interparietal bones, the width of the posterior border of the nasal bones, and the height of the rostrum were determined from camera lucida drawings. From these drawings, the interparietal areas were determined with a planimeter and the nasal posterior widths measured with a ruler at a point just above the suture line where the nasal bones are joined by the frontal. The rostrum heights were estimated in a region just anterior to the infra-orbital canal and the figures made comparable to

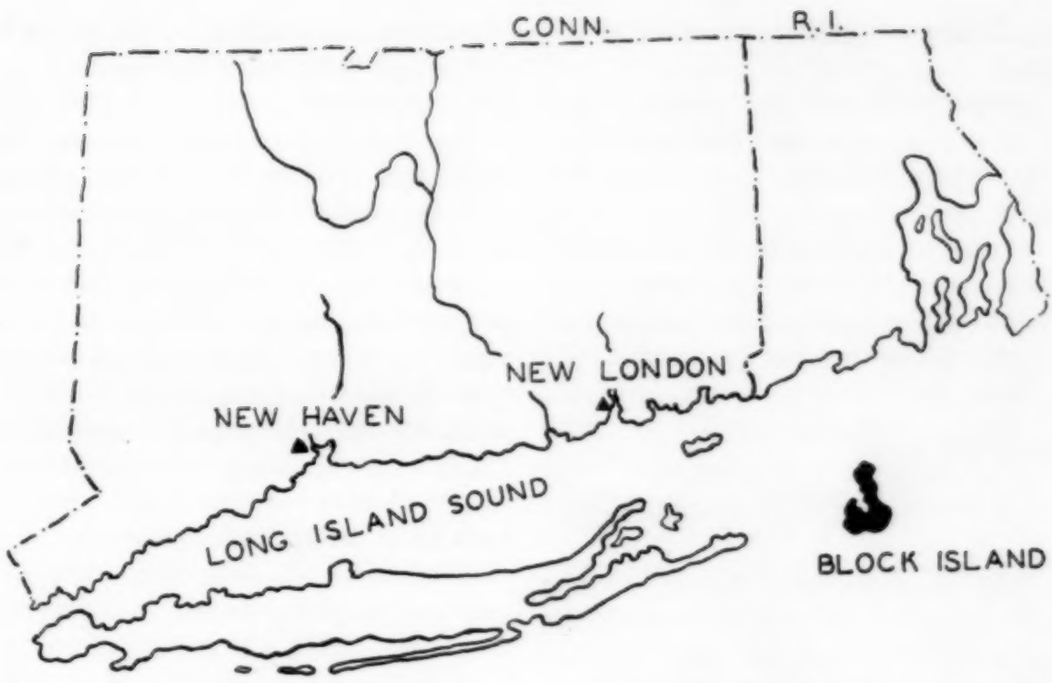

FIG. 1. Outline map showing the geographical location of Block Island in relation to Connecticut, Rhode Island and Long Island.

other measurements in the series made on the skulls directly. Silhouettes of the nasal and interparietal bones, traced from the original camera lucida drawings onto black paper, can be seen in figs. 3 and 4.

All data, when treated statistically, were considered to have been small samples.

EXPERIMENTAL RESULTS

Populations of Mainland and Island Microtus Compared

All Microtus representative of the mainland and island populations discussed in this section were trapped during the summer of 1949. Fifty-two mice were caught in the vicinity of New London during a period extending from July 6 through July 28. Forty-six specimens were taken from the Block Island population between July 31 and August 4. Although the trapping technique was not designed to determine population size or density, it is of interest to point out that in spite of only a six day trapping period on the island during which 405 snap traps were set, the number of insular mice caught was comparable to the number of specimens taken on the mainland where approximately 1400 traps were set during the 19 day trapping period.

Comparison of the distribution of sexes within each population showed some variation. The number of insular males and females closely approximated the expected 1:1 ratio whereas among the mainland mice, the females outnumbered the males almost 2:1. The sex distribution within the mainland sample may be attributed to greater wandering by the males; this difference might not appear among mice on the island where crowded conditions would tend to cut down the distances travelled.

Since body size was one of the criteria for the insular Microtus having been considered a separate species, it was of interest first to determine the mean body length of the mice within each population and secondly to establish the proportions of mice within 10 mm. size classes in both populations. It has been pointed out that all the mice for this part of the study were trapped during the same summer and all within a period of about one month. Therefore any variability in the proportions of the size classes within each population which might have resulted from

trapping during different seasons has been eliminated.

An examination of the mean body lengths of insular and mainland mice recorded in Figure 2 shows the means to be practically identical.

The degree of variability in the entire populations of both areas was determined by calculating the standard deviations for the two populations according to the following formula:

$$\sigma \text{ whole population} = \sqrt{\frac{N}{N-1}} \cdot \sigma \text{ sample.}$$

These data, recorded in Figure 2, indicate that body size is extremely variable and also that the mainland mice appear to be somewhat more variable ($\sigma = 15.01$) in respect to this characteristic than are the insular mice ($\sigma = 14.02$). Statistical analysis, however, did not show this difference to be significant ($P = < .8 > .7$). Much of this variability is undoubtedly due to age differences within the two sample populations.

The data in fig. 2 also indicate the frequency of mice in 10 mm. size classes for both populations. It is apparent immediately that all size classes are represented in both but a noticeable difference in proportions exists within the 110–120 mm. size class. Since the island population is almost certainly subject to less predation than the population on the mainland, it is tempting to suggest that the fewer number of mainland mice in this size class indicates a heavier predation on the mainland. It is of interest to mention that no shrews were ever taken in the traps set on Block Island whereas they were always caught in the mainland mouse runways. These mammals are one of the effective predators of field mice (Eadie, 1944) and the present experimental results might reflect the absence of

FIGURE 2

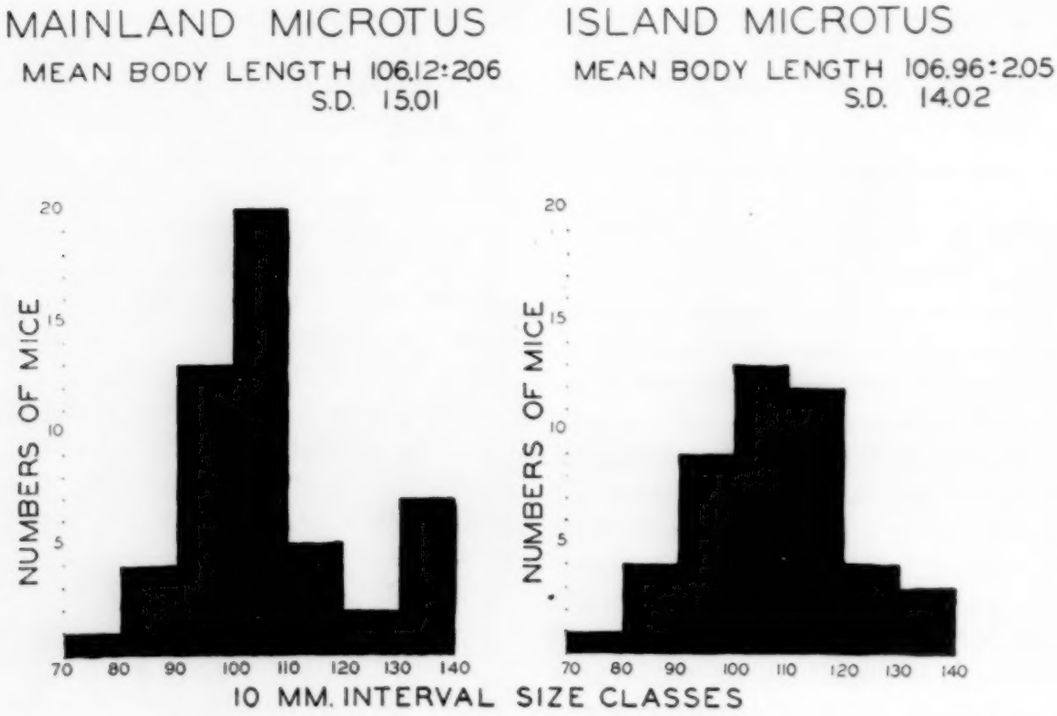

FIG. 2. Histograms indicating numbers of mice in 10 mm. size classes within both mainland and island sample populations.

Mainland sample N = 52; island sample N = 46.

Mean body lengths and standard errors of means of mice in each sample population given in millimeters.

S.D. = standard deviation. Standard deviation values are for entire populations.

shrews on Block Island assuming that the larger proportion of field mice destroyed by shrews on the mainland were in the 110–120 mm. size class. But Errington (1946) has carefully pointed out that variabilities in the size of a trapping area and the variation in the mobility of a species can easily distort analyses and it therefore seems preferable at this time not to attribute the scarcity of mainland mice in the 110–120 mm. size class to predation. Reservation of any explanation for the fewer mainland mice in this group should be exercised until data are collected under more controlled conditions; until the possibilities of variations due to sampling are eliminated, it is not profitable to attempt to explain proportion differences on the basis of ecological factors operating within the population, interesting as the speculations may be.

Measurements made on the tails, ears, and feet of both island and mainland mice failed to show any significant differences. The number of specimens available for these measurements was not in every case the total number of mice in each population because occasionally the ears were chewed before the specimens could be taken from the traps or sometimes the tails and feet suffered damage from the traps.

A Comparison of Mature Specimens from Block Island and the Mainland

Since the Block Island Microtus data which Bangs published was concerned with "adult" specimens only (Bangs, 1908), it seemed desirable to look for differences which might exist between mature specimens taken from both the mainland and the island.

Body size is a very dangerous if not completely inadequate criterion for determining maturity. Variation in body size can result from a number of factors and therefore one should not identify all smaller mice as younger members of a population or all larger specimens as older. It is obviously true that this correlation does exist within very broad limits but in determining maturity some criterion other than body size should be considered.

Within this study, several features of the skulls were used to establish the maturity of a specimen. In the skull of a fully mature animal, the temporal ridges are always apparent and almost always meet and fuse in the anterior region of the skull to form an interorbital crest. In addition, the interorbital constriction tends to become narrower with age and it is often possible to discern the tortuous courses of the sutures characteristic of adult specimens.

The 46 specimens taken from Block Island during the summer of 1949 comprised the island population from which mature animals were selected. The mature specimens of the mainland population were selected from Microtus trapped in the vicinity of New London and New Haven during a period extending from February 1949–March 1950.

Because the skulls were subject to considerable damage by the traps, the number of mature specimens analyzed is not necessarily indicative of the total number of mature animals within the populations. A specimen was never selected as being mature unless a large enough portion of the skull was available to determine maturity. Therefore data indicating the proportions of mature animals within each population are not available.

Before considering the data relevant to mature specimens, it should be mentioned that variability due to possible age differences has not necessarily been eliminated even among the so-called mature specimens. Without additional data to indicate the range of ages at which skulls of mice from both populations may exhibit "mature criteria," variability due to differences in age may be a factor which should be taken into consideration when comparing some of the morphological features of insular and mainland Microtus.

A tendency toward larger body size among mature animals within the island population is revealed by the data in table

TABLE 1. *Mean lengths of bodies, tails, ears, and feet of mature mainland and island Microtus*

	Mainland			Island		
	N	Mean length (mm.) and σm	σ	N	Mean length (mm.) and σm	σ
Bodies	16	116.25 ± 2.92	11.68	11	121.27 ± 4.19	6.81
Tails	15	42.33 ± .97	3.75	11	42.95 ± .12	.68
Ears (Base of pinna to tip)	10	11.50 ± .68	2.16	9	13.22 ± .14	.43
Feet (Left hind foot, plantar surface)	8	20.37 ± .53	1.50	11	23.36 ± .23	.78

1; this is in agreement with Bangs (1908). However, when treated statistically, the difference between the mean body lengths was not significant ($P = < .30 > .20$). The mean tail, ear, and foot lengths, also presented in table 1, although again indicating a tendency toward larger size in the insular Microtus, failed to reveal any statistical significance when the differences between them were analyzed.

In every case there is less variability for each of these characteristics among the island mice. Although statistical analysis failed to show a significant value for differences between the standard deviations except in the case of the tails ($P = < .02 > .01$) and ears ($P = < .03 > .02$), the consistently lower variability among the island mice in all these characteristics is of some interest. Although the greater variability of the mainland mice could be due to a greater diversity in age of the population sample, the data are certainly what one would expect when considering them from a strictly genetic view point. Possibilities for greater genetic homogeneity and therefore less variability do exist among the island mice due to the geographical isolation of this population.

Comparison of the Nasal and Interparietal Bones of the Skull

The skulls included in this analysis were selected on the basis of maturity using the criteria already described. Inasmuch as it was not possible to detect any differences between the skulls of mice trapped during the summer of 1949 on Block Island and those previously caught by Bangs from that area, the mature skulls from the Bangs' collection were included in this study.

Figure 3, which is composed of silhouettes made from camera lucida drawings of the nasal bones, shows, by inspection, a greater average posterior width among nasal bones of the island mice and when this width is compared statistically with similar measurements of mainland Microtus nasal bones, the difference between the two widths is significant; the island Microtus have a nasal posterior width which is greater than that of the mainland mice ($P = < .01$).

Figure 4 shows a striking difference between the sizes of the interparietal bones of the island and mainland Microtus. When the difference between the mean areas of these skull bones is compared, a highly significant size difference is apparent ($P = < .01$). Again, the island Microtus have a significantly larger interparietal bone than do the mainland mice.

It seems unlikely that these size differences in the nasal and interparietal bones are due to differences in age of the island and mainland population samples. Once certain characteristics appear which make it possible to designate the skulls of mice from both populations as mature, the relative proportions of the skull bones have become more or less fixed and a basis for

FIGURE 3

NASAL BONES

MAINLAND MICROTUS

MEAN POSTERIOR WIDTH 14.81±44 MM.
STANDARD DEVIATION 1.72

ISLAND MICROTUS

MEAN POSTERIOR WIDTH 18.73±36 MM.
STANDARD DEVIATION 1.49

FIG. 3. Silhouettes traced from camera lucida drawings of nasal bones from skulls of mainland and island Microtus.

Mean posterior widths and standard errors of means are indicated according to the magnification at which drawings were made (× 12).

comparison of the individual bones is possible without age differences complicating the picture. The present comparison of interparietal and nasal bones has been made after maturity has been established; beyond this point little change occurs in the skull bones. Variability in the size of these bones *within* one population may re-

FIGURE 4
INTERPARIETAL BONES

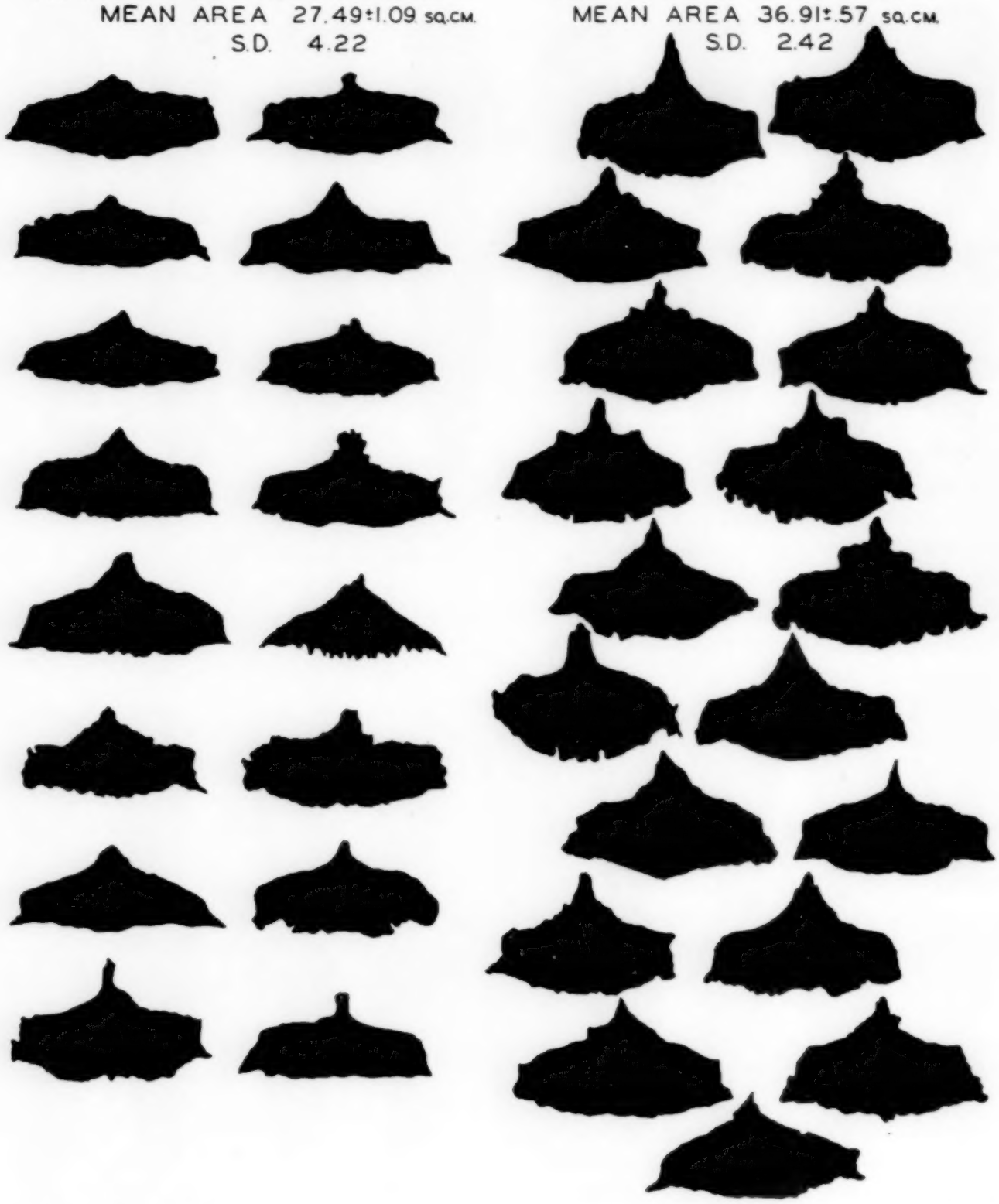

FIG. 4. Silhouettes traced from camera lucida drawings of interparietal bones from skulls of mainland and island Microtus.

Mean areas of the interparietals and standard errors of means stated according to magnification at which drawings were made (× 12).

flect diversity of age within the sample, but skull bone size differences noted *between* the two samples are not likely to be due to age differences. It therefore seems reasonable to suggest that these size differences are expressions of different genetic complexes.

Although statistical analysis of the differences between the standard deviations failed to show significant values, the variabilities of the interparietal and nasal bones are again consistently lower among the insular Microtus ($\sigma = 2.42$, $\sigma = 1.49$, respectively). Variability due to age diversity within a sample is still a possibility, but assuming that the skull bone pattern is genetically determined, these data may also be indicative of greater genetic homogeneity in the island mice.

Comparison of Measurements made on Entire Skulls

A series of linear measurements of various areas of the skulls was made in order to ascertain any additional size differences which might exist between the skulls of the island and mainland Microtus. These data are recorded in table 2.

The basal length of the skulls was taken from the basal occipital condyle to the posterior border of the incisor alveolus and the skull width was measured between the widest portion of the zygomatic arches just above the region where the jugal and maxilla join. The distance between the basal occipital condyle and the anterior superior border of the zygoma was taken as the condylar-zygoma measurement. By subtracting the condylar-zygoma measurement from the total basal length, a figure was derived indicating the length of the snout. The alveolar basilar measurement was the distance from the posterior border of the last molar tooth to the posterior border of the incisor alveolus. Rostrum height, as already indicated, was the only measurement in this series made from camera lucida drawings.

The number of skulls for each different measurement varies to some extent because although enough of the skull was extant to determine its maturity in every case, it was not always a complete skull. Depending upon where damage had occurred, the skull either could or could not be used for a particular measurement.

When the probability values for significance between means of these various measurements were determined, only the condylar-zygoma lengths and rostrum heights showed any significant differences ($P = < .01$ and $P = < .05$, respectively). In both these characteristics, the island Microtus showed larger proportions. It seems likely that the larger

TABLE 2. *Means of measurements of mature Microtus skulls from mainland and island populations*

	N	Mean (mm.) and σm	σ
Basal Length			
Mainland	13	26.33 ± .29	1.00
Island	16	26.76 ± .21	.80
Zygomatic Width			
Mainland	17	15.62 ± .14	.52
Island	16	16.00 ± .18	.71
Condylar-zygoma Length			
Mainland	14	21.36 ± .19	.69
Island	15	22.15 ± .13	.48
Alveolar-Basilar Length			
Mainland	17	15.79 ± .14	.57
Island	19	15.75 ± .14	.57
Rostrum Height			
Mainland	15	5.48 ± .08	.29
Island	18	5.73 ± .07	.29
Snout Length			
Mainland	13	4.98 ± .12	.43
Island	15	4.69 ± .10	.38
Upper Molar Tooth Row			
Mainland	18	7.01 ± .05	.23
Island	20	6.88 ± .05	.18
Diastema			
Mainland	16	8.75 ± .11	.50
Island	19	8.91 ± .12	.56

condylar-zygoma length in the island Microtus is a reflection of the larger interparietal. Assuming that the proportions of other skull bones anterior to the interparietal are the same in the island and mainland skulls, a significantly larger interparietal would tend to move the superior anterior border of the zygoma more anteriorly and thus create a significantly greater condylar-zygoma length in the skulls of the island mice; it is not possible to relate the broader rostrum in any way to the size differences in the posterior region of the skull. It is of interest to mention that these differences in the anterior region are evident when one examines the living animals; the mainland Microtus have a rather sharply pointed nose in contrast to the broader blunter nose of the Block Island mice.

Discussion

Since the length of time during which a population may have been isolated is an integral part of the speciation process, it is of interest to speculate, at least, how long the insular Microtus may have inhabited Block Island even though it is possible to make only an estimation. To arrive at any reasonable conclusion, both the means by which the mice first reached the island and the time at which the island vegetation provided a suitable niche must be considered.

Although Microtus are capable of adapting themselves to almost any environmental situation which provides suitable food, they find meadows of grasses and annuals the most favorable (Bailey, 1924). They do occur on the mainland in dense woods along with deer mice and shrews but do not become established in large numbers under these environmental conditions. Field mice also sometimes inhabit marshes and are often forced to swim short distances when their runways are flooded and may even take refuge on floating jetsam (Hamilton, 1940). Microtus from the mainland might have successfully spanned the distance to Block Island by riding on bits of driftwood but the journey would have been hazardous; the ocean currents between the mainland and the island are strong ones. It seems equally plausible to suggest that man might have been originally responsible for the transport of field mice to the island.

There are records indicating cattle transport to Block Island during the middle part of the seventeenth century and somewhat later (ci. 1700) due to denudation of the forests, the inhabitants of the island had been forced to erect stone fences constructed of glacial debris cleared from the fields (Livermore, 1877). It seems possible, therefore, that Microtus might have been transported as stowaways in cattle food supplies and a deforested terrain with open fields would have provided an optimum niche in which they could have become established. It is therefore suggested that the insular population of field mice was introduced on Block Island about 250 years ago.

A span of 250 years is a brief one but morphological differences of subspecific rank have been detected in the skull bones of the insular Microtus. As far as can be determined, the larger interparietal, the wider posterior border of the nasal bones, and the greater height of the rostrum in the insular Microtus skulls have no adaptive value. If the expression of these characteristics has a genetic basis, which seems likely, they may be a result of only relatively few genetic differences having been established by chance through inbreeding in an initially small insular population.

The question should be raised as to whether the small number of morphological differences between the island and mainland Microtus can be accounted for by reinvasions of mice from the mainland. If such were the case, one would expect fewer differences due to a break down in the isolation of the insular population, assuming that interbreeding between the two populations is still possible (Wheeler, 1952). Certainly the chances for reinvasion have become increasingly greater;

communication with the mainland has increased within the past 150 years and it is quite possible that a few mice from the mainland have reached the island during this period of time. But it seems unlikely that the few morphological differences can best be explained by genetic swamping from sporadic reinvasions. The island mice caught during the summer of 1949 were apparently identical to those collected by Bangs in 1899. If random invaders had had any pronounced genetic effect upon the insular population, it seems unlikely that consistent differences in the skull bones would persist after 50 years of genetic recombination.

Although the differences in body size of mature specimens may also be due to genetic diversity, these differences are not statistically significant and not too much emphasis should be placed on them in support of a distinct insular species of Microtus. It seems better to judge the species status of the Block Island mice on the basis of skull differences brought out in the current experimental results and of breeding data available in a former publication which indicated that hybrids resulted from a cross between a mainland female and an insular male (Wheeler, 1952). The fertility of the hybrids was not determined but the presence of the F_1's showed at least that sexual isolation was not complete and suggested that interbreeding was prevented in nature only through geographical isolation and not through fundamental physiological differences intrinsic within the two populations. The few morphological differences which exist between the mainland and island populations seem to substantiate the hypothesis that little genetic diversity has resulted since the island mice were established on the island. With the information which is available, it seems more reasonable to conclude that the insular Microtus have not become sufficiently distinct to be regarded as a separate species, but should be considered as an insular race, *Microtus pennsylvanicus provectus* Bangs.

Summary

The population of Block Island Microtus originally described as a separate species (*M. provectus*) shows few characteristics which are distinct from those of field mice on the neighboring mainland (*M. pennsylvanicus*).

No statistically significant differences in mean body lengths could be detected between the sample populations of mainland and insular Microtus.

Microtus selected from each population on the basis of skull characteristics indicating maturity failed to show any statistically significant body size differences. The mature insular mice, however, showed a tendency toward larger size not only in mean body length but also in the mean lengths of the tails, ears, and feet.

An analysis of the skulls revealed that both the posterior width of the nasal bones, the area of the interparietal bones, the condylar-zygoma measurements, and the height of the rostrum of the island Microtus skulls were significantly greater. It seems likely that the condylar-zygoma measurement is a consequence of a larger interparietal bone in the posterior region of the skulls of the island mice.

It is suggested, in view of the lack of significant differences between mean body lengths and the presence of only relatively few significant differences between the skulls of the mainland and insular Microtus, that the Block Island Microtus be considered an insular race, *Microtus pennsylvanicus provectus* Bangs.

Acknowledgments

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also wish to thank the Museum of Comparative Zoology at Harvard University for lending the Bangs' collection of Microtus study skins and skulls.

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EVOLUTION OF THE LOOP AND LOPHOPHORE IN TEREBRATULOID BRACHIOPODS

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INTRODUCTION

The contribution of paleontology to the study of evolution must come primarily through exposition of the actual course of organic development as revealed by fossil remains. Groups vary widely in the excellence of their fossil record, however, and it is evident that relatively few among the host of plant and animal lineages are well suited for phylogenetic study. Fortunately some groups, especially among marine invertebrates, have left records of superior quality. From among these the brachiopod order Terebratuloidea has been selected as the subject of this study.

The shells of terebratuloids (and apparently all other articulate brachiopods) consist exclusively of the relatively stable mineral, calcite, and thus tend to be well preserved. The existence of living representatives of the group has made possible investigations of the anatomy and of the relationship between soft and hard parts. It is known therefore that there is an intimate relationship between certain vital soft parts and some of the skeletal features. Further the group has an extremely long known history for it ranges from the higher Silurian to the present.[2]

Several of the skeletal features of Terebratuloids are known to undergo progressive change. The modifications of the loop are of the greatest interest because they reflect the nature of the lophophore and it is the purpose of this article to outline the historical development of the terebratuloid loop insofar as it is now known. While in general discussion is limited to the loop and associated soft parts, the nature of the cardinalia and in fact all available characters have been brought to bear in discovering the group relationships basic to this discussion.

An excellent phylogenetic study of the Silurian and Devonian terebratuloids has been published by Cloud (1942). Unpublished studies by the writer have followed the development of the group through the Mississippian, Pennsylvanian and Permian. The major courses of terebratuloid evolution during the Paleozoic are therefore known and it is possible to consider developments in the loop with some confidence.

Unfortunately the Mesozoic representatives of the group are less well known. The most serious gap in our knowledge of terebratuloids exists among Triassic forms. For the Mesozoic therefore only the broad outlines of loop development can be suggested. Cenozoic forms are again better known and the majority of genera persist in recent seas.

The loop can not be reasonably treated simply as a skeletal structure, but must be related to the associated soft parts. Neither can it be studied only in the adult for in many terebratuloids it passes through a complex ontogenetic history. Before an examination of the fossil record can be profitably undertaken, therefore, the bearing of certain zoologic evidence must be considered.

LOPHOPHORE AND LOOP OF MODERN TEREBRATULOIDS

Lophophore Development

Modern terebratuloids are currently placed in two superfamilies, the Tere-

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[2] The most recent data on absolute time datings indicate that this interval represents ca. 3.1×10^8 years.

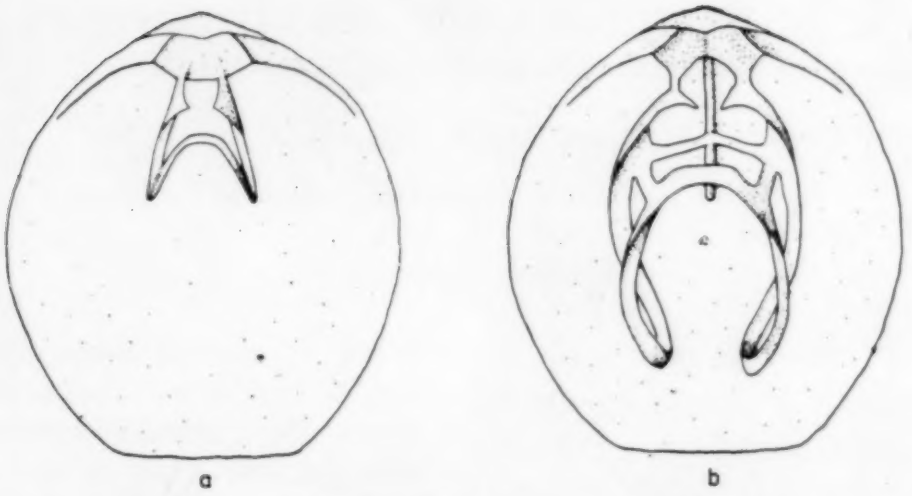


FIG. 1. Loops of recent terebratuloids. a. Schematic representation of the short loop characteristic of terebratulaceans and its relationship to the dorsal valve. b. Schematic representation of the long loop characteristic of terebratellaceans and its relationship to the dorsal valve.

bratulacea and Terebratellacea. The most apparent difference between the two is found in the nature and development of the loop. Among the Terebratulacea the loop is short and simple and develops directly from the cardinalia (fig. 1a). In the Terebratellacea the loop is long and complex and arises in part from a median septum (fig. 1b).

While the loops differ widely in the two superfamilies, it is interesting to note that the gross structure and ontogeny of the lophophore in typical members of each is similar. In characteristic forms in

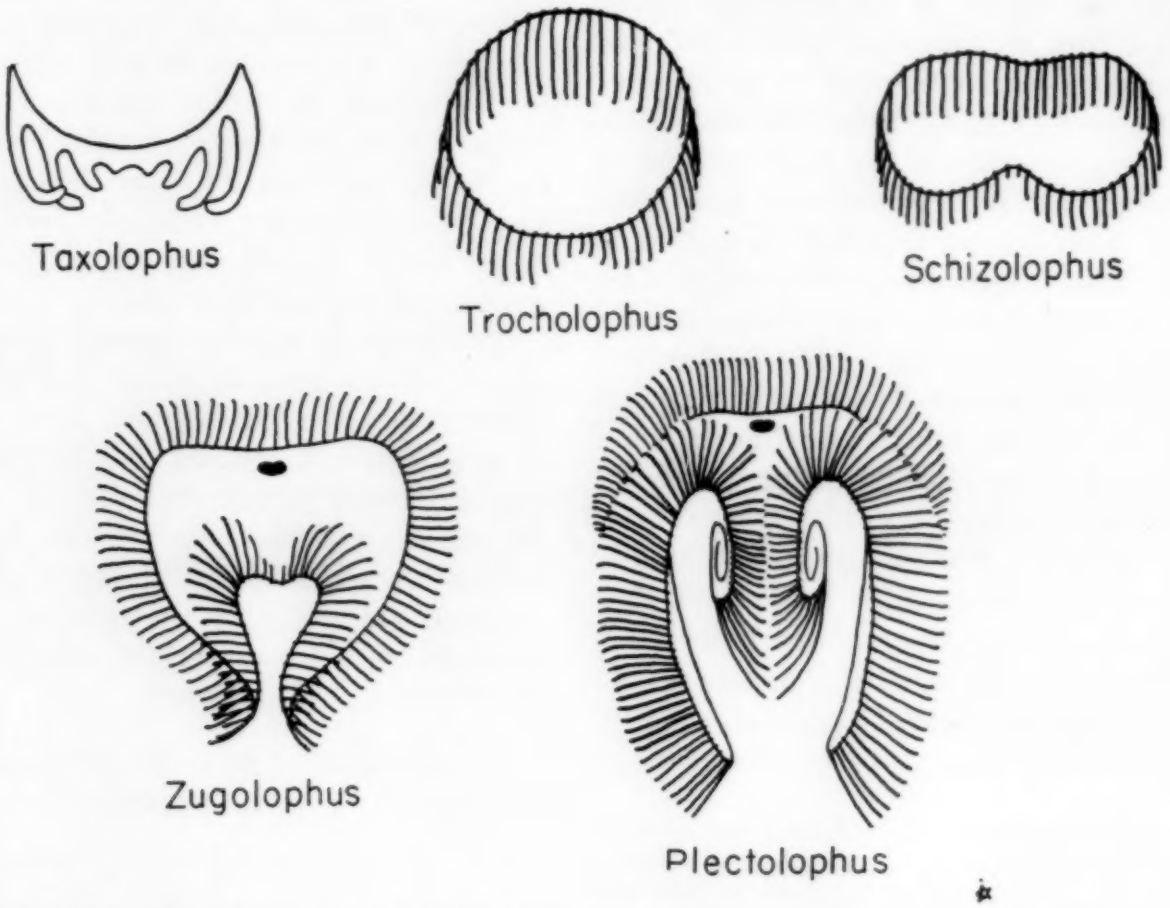


FIG. 2. The ontogeny of the lophophore showing progressive developmental stages from the initial Taxolophus stage to the final Plectolophus stage (after Thompson).

both superfamilies the lophophore is initiated as a simple semi-circular band about the mouth. As growth proceeds the lophophore grows longer, becomes medially recurved and finally becomes divided into a "lateral arm" at either side and a median pair of "spiral arms" (fig. 2). This last condition, spoken of as plectolophus, is characteristic of the adults of most genera.

Terebratulacean Loop

With the nature of the lophophore in mind we may examine its association with the supporting calcareous loop. The degree of support afforded by the loops of the two superfamilies is widely dissimilar. Among the Terebratulacea the lophophore is supported by the loop only at its base (fig. 3a). The mouth enters the body wall between the crural points of the loop which support the primary, descending bands of the lophophore in this region. The lophophore then follows the main band of the loop to the termination of that structure and extends far beyond it. The recurving bands of the lateral arms rest, near their base, on the transverse band of the loop toward its lateral margins. The spiral arms rest basally on the median portion of the transverse band of the loop, where their position is often indicated by a notch. They then pass forward into the spiral which lies antero-dorsal to the loop and touches it only where the first turn of the spiral lies against the dorsal side of the transverse band. Perhaps because of the inadequate support furnished by the loop the lophophore is commonly strengthened by abundant calcite spicules within its tissues.

The ontogenetic development of the loop and lophophore has been studied in the modern, *Terebratulina* (Morse, 1871). It has been shown that while the lophophore passes through the stages seen in figure 3, the loop first appears in essentially its final form and is subsequently significantly altered only by growth in size. In other modern representatives the development, insofar as known, is similar.

Terebratellacean Loop

Among the Terebratellacea the plectolophus lophophore of the typical adult arises, as in the Terebratulacea, at either side of the mouth and passes along the crural points (fig. 3b). The primary descending bands of the lophophore then follow the main bands of the loop which furnish complete support. The recurving bands of the lateral arms follow the recurved bands of the loop which again

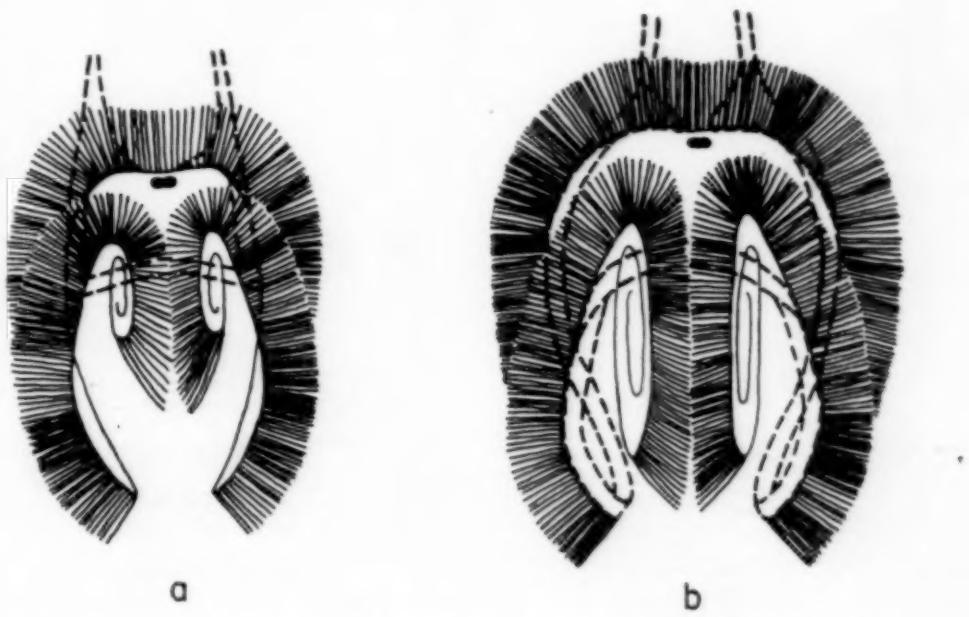


FIG. 3. Relationship of the loop and lophophore in recent adult terebratuloids (loop shown by dashed lines). a. A short (terebratulacean) loop and the associated lophophore. b. A long (terebratellacean) loop and the associated lophophore.

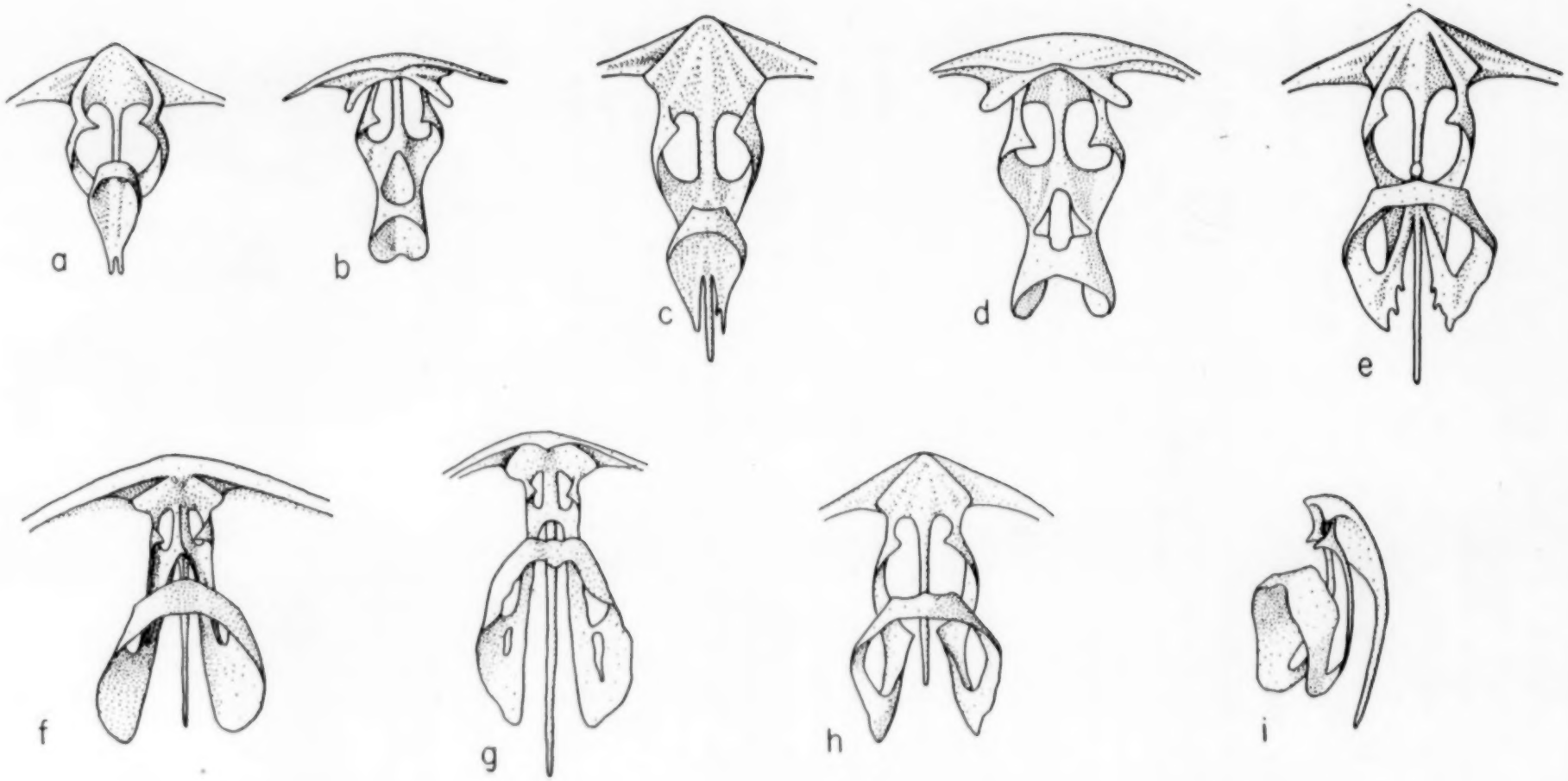


FIG. 4. Metamorphosis of a terebratellacean loop during ontogeny (*Dallina septigera* after various authors). Progressive stages shown from a–i. a, c, e and h as seen from a position normal to the plane of the loop. b, d, f and g as seen with the valve somewhat rotated clockwise to show changing nature of connection with the median septum. i shows the adult loop completely freed of the septum, as it appears in profile.

furnish complete support. The lophophore then enters the spiral arm and is supported by the transverse band upon the ventral side of which it rests. The first turn of the spiral rests against the dorsal side of the transverse band and thereafter the lophophore receives no further support. The lophophore is in general well supported by the loop and spicules are seldom if ever developed.

The ontogeny of the loop and lophophore have been studied in several genera by a number of investigators. The lophophore passes through the stages shown in figure 2, but the loop has a complex developmental history. It develops in part from the cardinalia and in part from a median septum which is universally present at least in the early growth stages of this group. The normal adult loop shown in figure 1a develops as a result of metamorphosis (fig. 4).

The details of loop ontogeny vary somewhat between various terebratellacean groups. Elliott (1953) for instance has distinguished three major modes of loop development, and notes that the developmental pattern shown by the loop is evidently less conservative than that of the lophophore. Association of the loop and the lophophore seems to be somewhat imperfect in the earlier developmental stages with growth of the two structures proceeding at unequal rates. A plectolophus condition of the lophophore is attained considerably before the final condition of the loop, designed for its support, is reached.

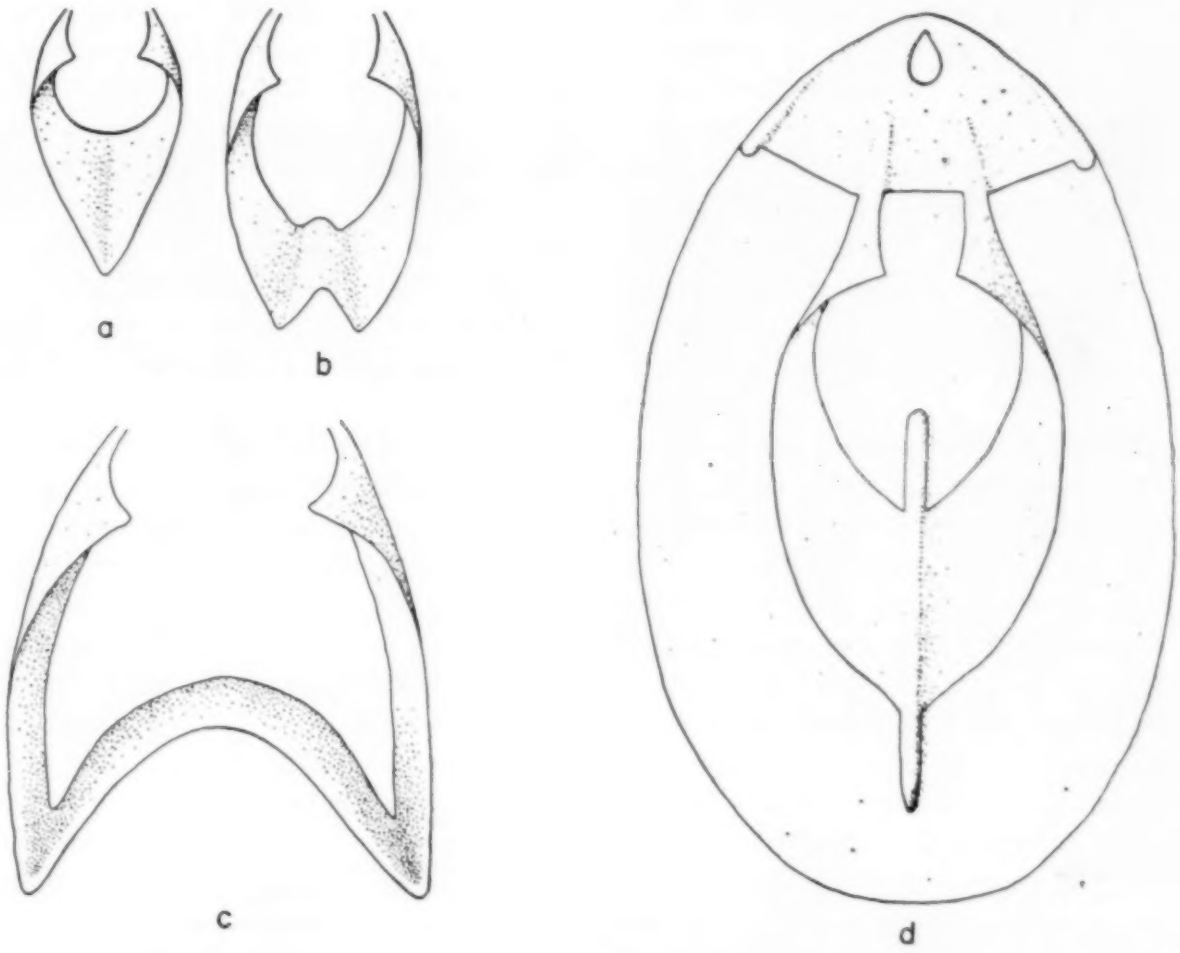


FIG. 5. Terebratuloid loops. a, b, and c. Three stages in the loop ontogeny of the Middle Paleozoic terebratulacean, *Cranaena*. a. The initial centronelliform stage. b. An intermediate stage. c. The adult short (terebratulacean) loop. d. The adult loop of a typical Middle Paleozoic centronellacean showing the centronelliform loop characteristic of the earliest terebratuloids.

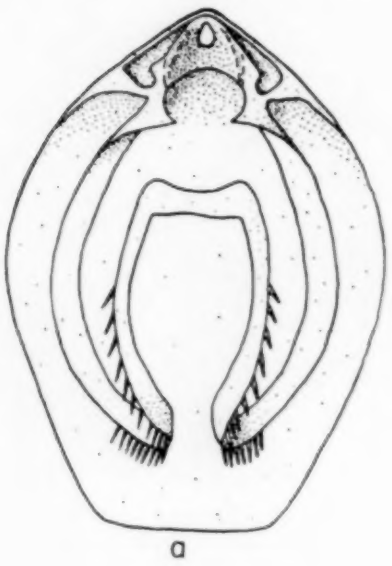
Loop Development Among Fossils

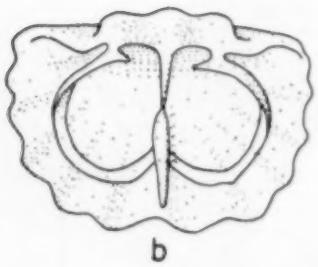
The Terebratulacean Loop

The loop sequences of the two modern terebratuloid superfamilies are best examined separately. The Terebratulacea is the more compact group and more easily dealt with so that it can be investigated first.

Fossil forms possessing all of the essential features of the Terebratulacea are known from high Lower Devonian rocks. We may drop back to this point in the stratigraphic sequence without further detailed discussion, but from here we must proceed downward in the rock succession more cautiously. By abruptly dropping back to the Devonian, I do not mean to suggest that the Terebratulacea were static during the intervening period. I simply reverse consideration of evolutionary events during this interval for later discussion.

The earliest obviously terebratulacean genus is *Cranaena* which has the typical loop of this superfamily and in addition beak characteristics and cardinalia which can be traced through their subsequent development into the modern condition. The loop (fig. 5a, b, c) is almost identical to the loops of modern plectolophus terebratulaceans. It shows none of the loop modifications which in a few paedomorphic modern genera accompany adult retention of a simple lophophore. Careful investigation of an excellently preserved





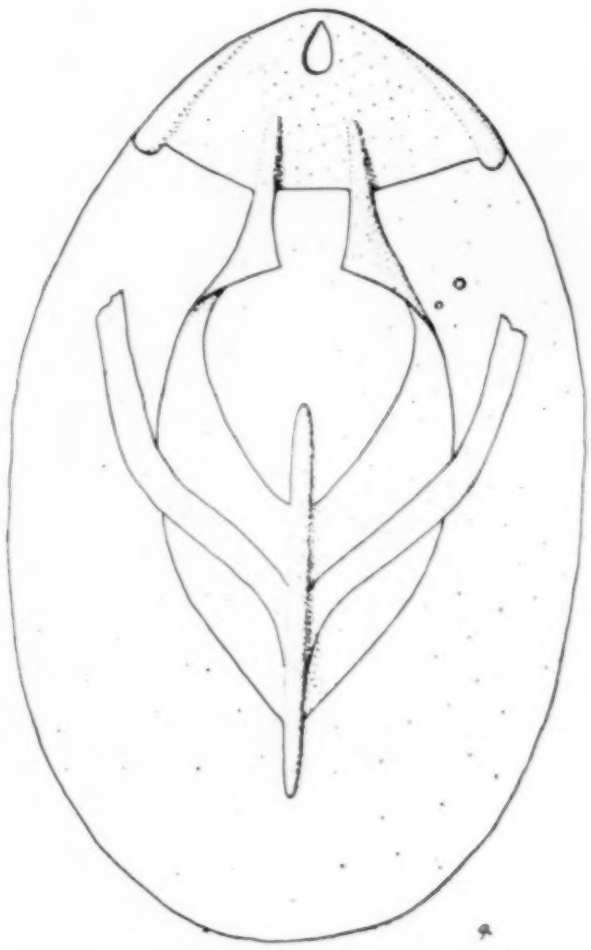


FIG. 6. Terebratuloid loops. a. Adult cryptonelliform loop of the type found in the Paleozoic and Mesozoic. This is a long loop differing from that of the more typical terebratellaceans in its failure to develop in part from the median septum during ontogeny. b. The essentially centronelliform loop of the adult of the living genus *Argyrotheca*. c. An extreme modification of the terebratulacean loop seen in the Permian genus *Gefonia* in which a paedomorphic centronelliform loop found in the adults of some closely related genera has been modified in the direction of a long loop by the development of recurving bands.

growth series in *Cranaena* has revealed that the loop ontogeny is unlike the simple development seen in the modern *Terebratulina*. The loop in its earliest stages is not simply a small replica of the adult form; rather a metamorphosis takes place. In its early growth the loop of *Cranaena* is centronelliform (compare fig. 5a, b, c, and 5d). This stage has evidently been suppressed by skipping in the ontogeny of modern forms.

The presence of a centronelliform loop in the ontogeny of *Cranaena* suggests that the ancestral stock may have had centronelliform adult loops. We may at this point consider the nature of such fossil terebratuloids as preceded the appearance of *Cranaena*. As it happens only two major loop types are known in older terebratuloids, one of these slightly and the other considerably predates *Cranaena*. The type which occurs only a little earlier is the cryptonelliform loop (fig. 6a) which is so specialized that we need not, at the moment, consider it further.

The other type is the centronelliform loop which characterizes almost all early terebratuloids and apparently all of the oldest ones. Both ontogeny and sequence in time suggest, therefore, that the ancestor of *Cranaena* bore a centronelliform loop.

Having traced the Terebratulacea into the basal terebratuloid complex of centronelliform looped types, let us defer further consideration of ancestry and instead follow the lineage from *Cranaena* to modern forms. Our concern here is principally with the evolution of the loop and lophophore. In general, the loop at least, and the lophophore insofar as we can infer, were extraordinarily stable in this lineage, despite the fact that other structures changed apace. Loop development can be followed through a number of genera until the Permian before any change is noted. In the Permian a number of genera having centronelliform adult loops are known. Other characteristics leave no doubt that they are nevertheless terebratulaceans, and if it is recalled that a centronelliform loop stage characterized the ontogeny of ancient terebratulaceans, it will be recognized as probable that the adult centronelliform loop is the result of paedomorphosis. The loops in these seemingly paedomorphic Permian genera tend to become very long, and they are also generally characterized by the development of a large vertical plate at the meeting of the two main bands, a feature that was common also among the older and primitive centronelliform loops.

It becomes important here to consider the significance of the retention of a centronelliform loop in the adult stage as it relates to the nature of the lophophore. Unfortunately, as noted earlier, the ontogeny of modern forms does not provide a centronelliform stage which might be used for comparison. Neither does any modern adult terebratulacean possess an adult centronelliform loop. We must therefore look elsewhere for evidence. The terebratellacean genus *Argyrotheca* possesses a loop which while somewhat peculiar in detail is basically centronelliform (fig. 6b). The associated lophophore is schizolophus. With this as a starting point we may proceed and other evidence of the looplophophore relationships may be added in the course of the discussion.

The space requirements of a fully developed plectolophus lophophore are not consistent with a centronelliform loop. In order to accommodate such a lophophore the loop must be open anteriorly for the reception of the spiral arms. The lophophore associated with a centronelliform loop therefore may be assumed to have been in a simpler condition. It was probably schizolophus as this best fits the form of the loop. The median incision of the lophophore would have lain upon the vertical plate while the main bands supported the remainder.

Let us assume for the moment that the lophophore of these centronelliform looped Permian terebratulaceans was schizolophus and follow the further developments of the loop. It has been noted that

the vertical plate in these genera was very high and was probably accommodated in the incision of a schizolophus lophophore. In *Gefonia* and in an undescribed genus recurved bands develop from the vertical plate and extend posteriorly into the mantle cavity (fig. 6c). These structures can be meaningfully interpreted only as lophophore supports and when so considered are seen to require the presence of a zugolophus or incipiently plectolophus lophophore. Both the persistent closure of the loop anteriorly and the absence of a transverse band show that the lophophore can hardly have reached full plectolophus development.

It is apparent that the specializations involved in the original fixation of the short loop in the terebratulaceans made further development unlikely as the lophophore was in large part unsupported by the loop and provided with independent spicular support. Further development of the loop could only be attained by, in a sense, starting over from the beginning. This was actually possible in the paedomorphic line and it is most interesting that evolution in this second chance follows the course originally chosen by the cryptonelliform looped terebratuloids in the Devonian.

Unfortunately our knowledge of Mesozoic and especially of Triassic terebratuloids does not yet allow complete unraveling of the post-Paleozoic fate of this unusual group of terebratulaceans. They are known, however, to persist in considerable abundance through most of the Triassic. It may or may not be significant that all of the Triassic forms known have simple centronelliform loops. None are known to show the recurved bands seen in Permian forms, but these could easily be overlooked.

It should not be overlooked that we have, in this group, during the Permian, less than a complete geologic period before the appearance of the first undoubted terebratellaceans in the Upper Triassic, forms which are evolving in the direction of a long loop. Only continued progress toward the development of a long loop and plectolophus lophophore, and the development of a median septum would be necessary to produce the internal features considered diagnostic of the Terebratellacea. A median septum might be developed either from a sessile cardinal plate or by modification of the vertical plate of the loop and its union with the floor of the dorsal valve. It may be noted here that *Aspidothyris* from the Carnian of Spiti, India, has not only a short median septum but also a vertical plate which seemingly unites with the dorsal valve. This genus, however, may actually be an early terebratellacean rather than a paedomorphic terebratulacean, and in any event requires restudy when material becomes available.

While it is conceivable that the loop of one of the paedomorphic terebratulaceans may have evolved into the type characteristic of the Terebratellacea other characteristics must also be considered. The cardinalia, commonly useful in determining relationships within the Terebratuloidea, are of little use here, for the cardinalia in each of the three groups which might be considered Terebratellacean ancestors are similar. Beak characteristics provide the only useful clues in the present instance. The beaks of Paleozoic terebratulaceans, including those believed to be paedomorphic, are characterized by a mesothyrid or permesothyrid, labiate or marginate pedicle foramen. Internally a pedicle collar is almost invariably present and well developed. Neither of these conditions is found in characteristic early terebratellaceans, but both persist in generalized modern terebratulaceans. It may be reasonably concluded, I think, that while further investigation of these forms is highly to be desired they do not include the ancestors of the Terebratellacea.

To return now to the conservative terebratulacean stock, we find that they continue in large numbers through the Permian and Triassic. The loop of *Coenothyris* is incorrectly illustrated in Zittel (1913), and investigation shows it to

have normal terebratulacean features. Jurassic forms are imperfectly known. By Cretaceous time however, a number of modern terebratulacean genera had appeared, along with many others. Almost all of the modern genera were present by the Eocene.

Some of the living genera reach their adult lophophore development before reaching the plectolophus stage. Lophophore development in these forms stops with the trocholophus or schizolophus stage. It is interesting that the associated loops are peculiarly modified but not centronelliform. This suggests that skipping eliminated the centronelliform stage in loop ontogeny by the time paedomorphosis occurred. As some paedomorphic modern genera are said to extend back to the Cretaceous, it is probable that skipping had occurred by that time. The ontogeny of fossil forms is in general poorly known but at least some of the Permian terebratulaceans (such as *Dielasma* ss.) do still possess an early centronelliform stage.

The Terebratellacean Loop in Fossil Genera

Leaving this suggested Devonian to Recent terebratulacean loop development, we may again drop backwards in time, but now along the terebratellacean family tree. True terebratellaceans have long been known from rocks as old as Middle Jurassic and Stehli (in press) has recently reported a late Triassic form from Peru. As typical terebratellacean are not known to occur in the Paleozoic, it appears probable that the group, at least as commonly defined, arose during the Triassic. As noted above, however, Triassic terebratuloid faunas are badly in need of comprehensive revision.

Aside from the Terebratulacea one possible centronellacean and undoubted Terebratellacea the only Triassic terebratuloids positively known are the genera constituting the Zeilleriidae. This group comprises forms having a median septum and a long, cryptonelliform loop. The Zeilleriidae may differ from the typical Terebratellacea in the failure of the loop to unite in ontogeny with the median septum, but careful investigations by Muir-Wood (1934) and unpublished studies by G. A. Cooper have shown that traces of a connecting band between the loop and the median septum exist in immature specimens of some Middle Jurassic genera which have been called Zeilleriids. Immature specimens of a Permian or Triassic form investigated by me however, show an immature centronelliform loop but do not show a connection with the septum. The Zeilleriidae are probably transitional between ancestral forms and the more typical terebratellaceans.

Because paedomorphosis appears to be common among modern terebratellacean genera and is believed to have been important in the evolution of the related Terebratulacea; and in view of the fact that such evidence as exists points in this direction, it is suggested that the Terebratellacea arose from the Zeilleriidae by paedomorphosis. A centronelliform loop is present in the very early ontogeny of primitive Zeilleriidae. It is supposed that this condition was extended into ontogeny and that the loop united with the median septum. This would have been highly advantageous to the animal, for loop development could now proceed from both the cardinal margin and the median septum, and larger size of the loop and lophophore could be attained more rapidly. Further advantage might be gained by a continued paedomorphic trend producing a loop either separated from the median septum only in the adult or remaining permanently attached. Paedomorphosis appears to have continued or perhaps been revived in various terebratellaceans to different degrees. It may have been a dominant factor in their diversification and, it is here suggested, in their origin as well.

In view of the strong possibility that the Zeilleriidae gave rise to the Terebratellacea, it may be of interest to consider the group further. As noted above,

it appears early in Triassic time. No definitely Permian forms are known to possess the median septum characteristic of the group but forms otherwise similar which have identical cryptonelliform loops are well known in the Permian. The Mississippian genus *"Harttina"* has all the essential features of the Zeilleriidae and may be either an early member of that group or a parallel development. It seems clear, in any case, that the Zeilleriidae are Mesozoic derivatives of the Paleozoic Cryptonellidae.

The Cryptonellidae are persistent but never abundant nor very diverse through much of the Paleozoic. *Cryptonella* itself, appearing in the Lower Devonian, is the oldest known genus in the lineage. Externally, especially in beak characters, *Cryptonella* is very similar not only to later Paleozoic cryptonellids, but to the zeilleriids and to many of the less specialized modern terebratellaceans. Internally it differs importantly from the zeilleriids only in the lack of a median septum and in this, plus its attachment of loop and septum, from terebratellaceans.

All terebratuloids in which the nature of the loop is known and which are sufficiently ancient to have been ancestral to the cryptonellids have centronelliform loops. As noted above, late Paleozoic or early Mesozoic zeilleriids at least, had a centronelliform stage in loop ontogeny so, as with the terebratulaceans, both ontogeny and sequence in time point to an origin in the centronelliform looped complex of early terebratuloids. While no genera actually transitional between *Cryptonella* and any earlier form are known structural parallels of the intervening steps may be seen in the ontogeny of another group.

Loop Evolution in the Centronellacea

In both the Terebratulacea and the Terebratellacea we have traced the lineages to the earliest genus definitely assignable to them. In each case the trail was lost in the Lower Devonian where the lines merged with the complex of forms involved in the archaic terebratuloid radiation. It is now appropriate that we should examine the loop and probable lophophore of these early forms.

As in any vigorously radiating group, these early terebratuloids show considerable variation. While this variation is apparent in all morphologic features, it is particularly evident in the loop. The basic plan of the loop in this group, which I propose to call the Centronellacea,[3] is centronelliform. As has been pointed out previously the lophophore associated with the centronelliform loop was probably schizolophus or even simpler. In view of the relative inefficiency of this lophophore under most conditions, it is not surprising to find that the dominant theme of loop and lophophore evolution in the Centronellacea is in the direction of increased complexity and efficiency. This is made doubly apparent by the fact that the only truly successful groups to emerge as persistent stocks from this radiation were those which proceeded furthest in lophophore development.

It seems pointless to pursue the details of the various short lived variations in loop development because of their great number. It may be sufficient to note that these apparently less successful groups within the Centronellacea are generally characterized by retention of the centronelliform loop which was frequently expanded to considerable size and elaborate detail. The longer ranging groups appear to have moved in the direction of a plectolophus lophophore. However, this in itself seemingly gave no assurance of "success," for several stocks which made

[3] The writer does not agree with Cloud (1942) that the presence or absence of crural plates is of great value in classification of these animals, and feels that the early radiation and its derivatives other than the successful Terebratulacea and Terebratellacea are more naturally treated as a single diverse superfamily. Unfortunately the evidence which has forced this conclusion is too voluminous for inclusion here, and is not immediately pertinent to the present discussion. It will however be presented elsewhere at another time.

early advances in this direction were short lived.

For the most part the centronellacean radiation was at an end by the end of the Middle Devonian and we may largely confine ourselves to those lines which survived into the Late Devonian and later time. Only one such line appears to be of real importance and this is more for the light it casts on loop evolution than for its own limited success. This group has as yet received no formal name and only two of its genera are named though at least four are known to be included. In external characteristics the group is extraordinarily compact, all of its members being characterized by the possession of a dorsal sulcus and ventral fold.

To judge from external characters this group is already present in the Middle Devonian (*"Centronella" alveata* Hall) but as a single specimen is known its internal structures could not be investigated. Serial sections of an unnamed Mississippian species show however a long centronelliform loop which departs from the basic plan only in that the vertical plate becomes divided into two at its ventral edge (fig. 7a). The loop is similar in this respect to the modern terebratelloid *Magadina* which possesses a zugolophus lophophore. It is most probable that the loop of this Mississippian form indicates development of a simple zugolophus lophophore from a schizolophus one.

A younger genus in this lineage occurring in Pennsylvanian rocks shows some advances in loop structure. Here the main bands unite, but only briefly, and then diverge anteriorly. From the anterior ventral margins of the main bands rises a small and hood-like but complete recurved band (fig. 7b). A descendant genus, *Cryptacanthia,* has a similar loop except that the main bands no longer unite and the recurved bands are more pronounced (fig. 7c). Both loops were probably associated with a well developed zugolophus lophophore though in *Cryptacanthia* an early plectolophus stage may have been reached.

Glossothyropsis from the Permian appears to represent the terminal member of this lineage (unless some of the species assigned to *Aulacothyris* in the Mesozoic belong here). In its loop the main bands are widely separated. The recurved portion of the loop is long and well developed (fig. 7d). The lophophore was almost certainly of a completely developed plectolophus type. A median septum is present in *Glossothyropsis* and though the loop is not known to be united with it at any stage, it could represent a potential ancestor for the Terebratellacea. The peculiar and characteristic external form of this lineage suggests, however, that it was not ancestral and in fact no Mesozoic representatives are surely known. The group is of significance rather because of the illustration it affords of the development of a long loop and plectolophus lophophore from a centronelliform loop

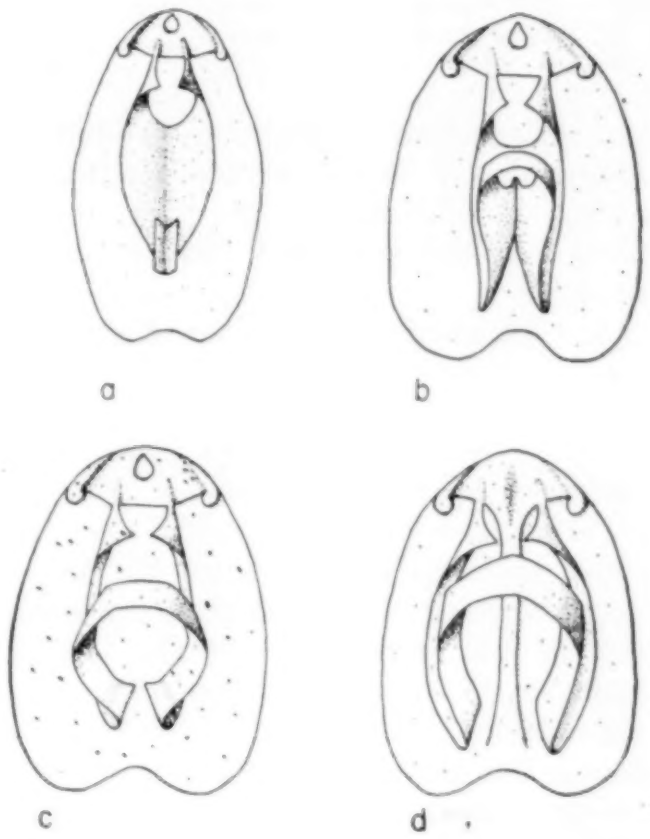


FIG. 7. The development of a cryptonelliform loop from a centronelliform loop as seen in four closely related and chronologically arranged Paleozoic centronellacean genera. a. A Devonian to Mississippian genus. b. A Pennsylvanian genus. c. A Pennsylvanian and Permian genus, *Cryptocanthia.* d. A Permian genus, *Glossothyropsis.*

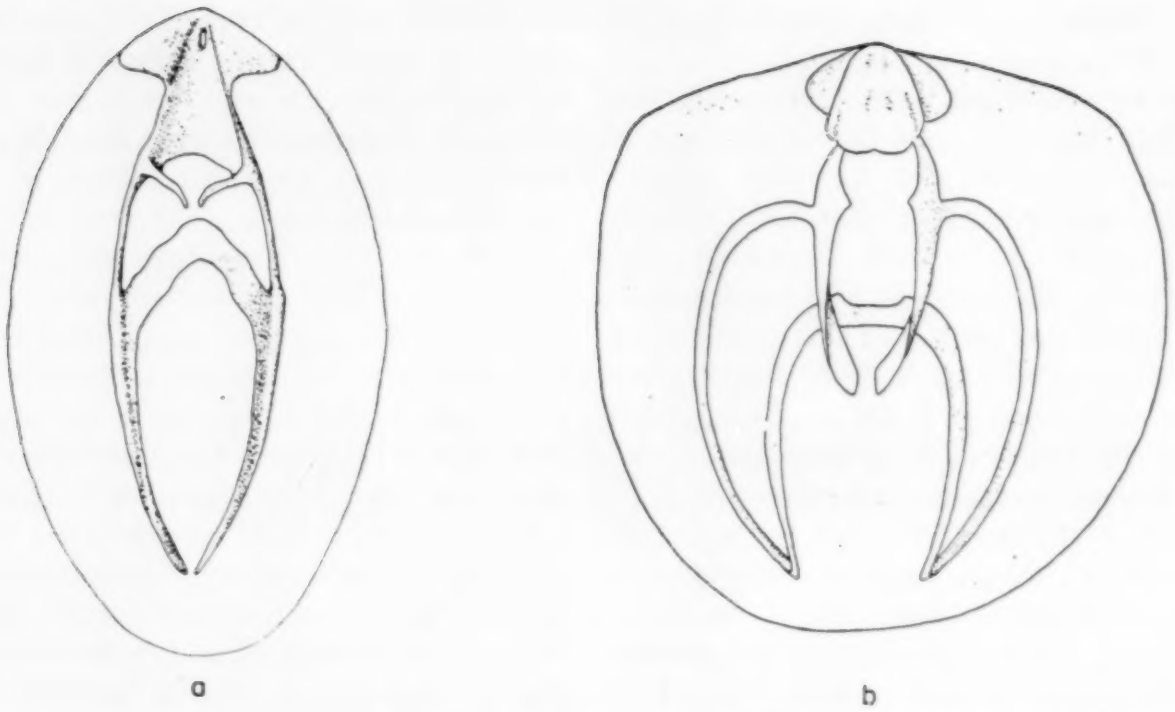


FIG. 8. Short lived centronellacean stocks of the Middle Paleozoic which independently developed long loops. a. *Cimicinella* from the German Lower Devonian in which recurved bands have developed but failed to separate from the main bands (after Schmidt). b. *Meganteris* from the Lower Devonian showing the development of a complete long loop which proceeded farther in the direction of lophophore support than in any living genus by developing from the crural points partial supports for the spiral arms (after Suess).

and schizolophus lophophore. The same steps probably intervened between an ancestral centronellacean and *Cryptonella* though in this case they have not been recorded.

As mentioned earlier other and less successful attempts at the production of a long loop occurred among the Centronellacea. *Cimicinella* from the Lower Devonian of Germany affords one example. In this genus the main bands have become separated from one another and a long recurving portion of the loop is developed but never becomes free of the main bands (fig. 8a). Another interesting sideline is seen in *Meganteris* also a Lower Devonian form. Here a well developed cryptonelliform loop is produced and the structure proceeds farther in the direction of lophophore support than any other known fossil or modern loop (fig. 8b). From the crural points processes develop which obviously functioned to support the initial portion of the spiral arms of a plectolophus lophophore.

A few generalized forms with typical centronelliform loops persisted into the middle Mississippian, but died out without descendants. They may be distinguished from paedomorphically centronelliform looped Terebratulaceans by the nature of the beak of the ventral valve and by the cardinal plate.

SUMMARY

The brachiopod order Terebratuloidea is a compact and long ranging group which has persisted through about 310 million years. Internally, its members are characterized by a delicate calcareous structure, the loop, which supported a food gathering organ, the lophophore. The evolutionary developments of the loop can be objectively and more or less continuously traced in the fossil record. Evolution of the associated lophophore can be inferred with considerable confidence from the structure of the loop and by analogy with living forms. The loop is a conservative structure and of great

value in recognition of relationships. Only the loop and lophophore are considered here but all available characters have been evaluated in reaching the classification basic to this discussion.

It is concluded that a centronelliform loop is normally associated with a schizolophus lophophore. Evolution of the lophophore proceeds in the direction of greater complexity (plectolophus) in the primitive members of all successful lineages and the steps involved in its attainment are similar in all groups. The loop evolved in response to the need for sup-

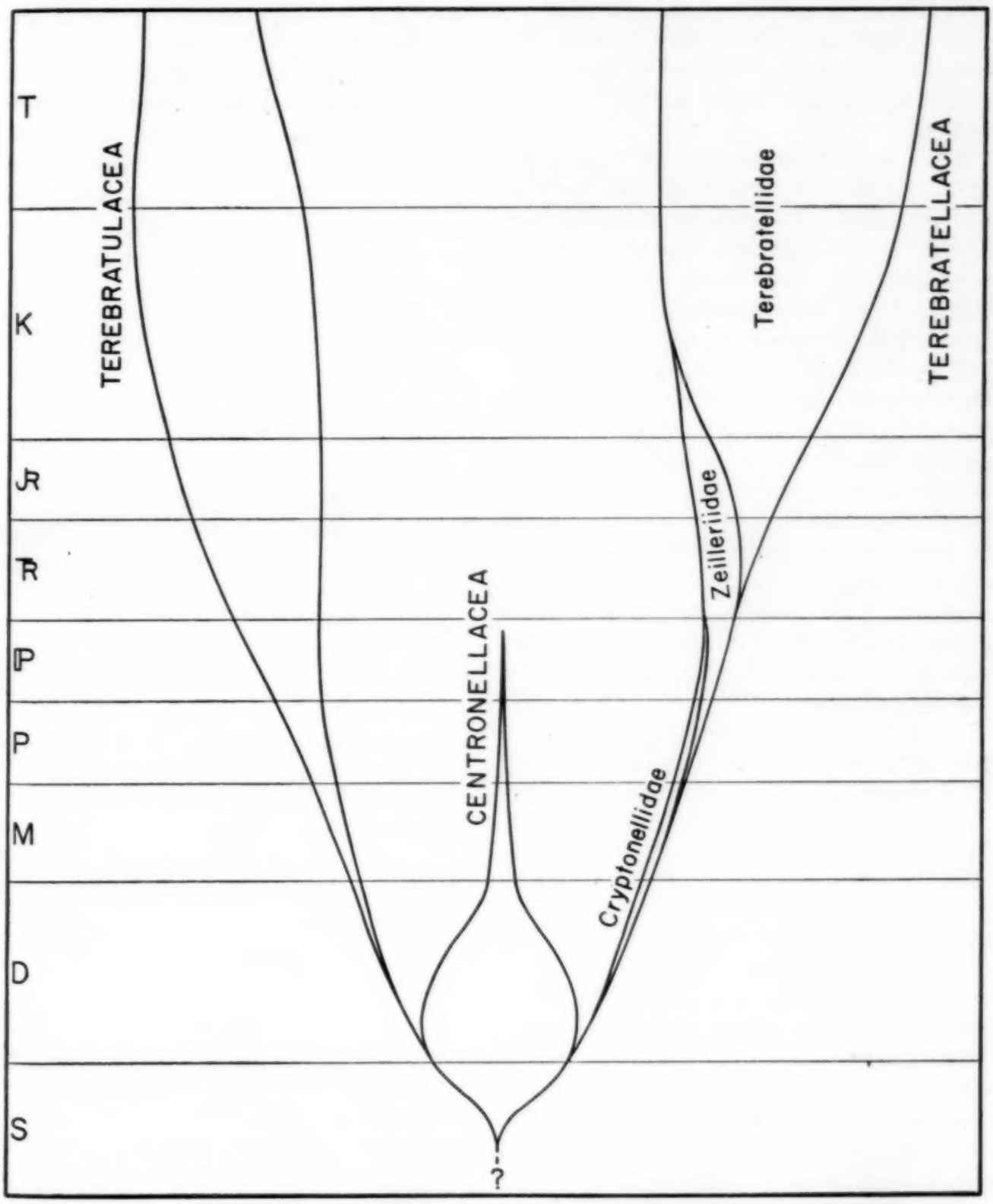


FIG. 9. The probable relationships of some important groups of the Terebratuloidea as they now appear to the writer on the basis of the evidence presented here, and additional unpublished data.

port of a progressively more complex lophophore.

It is felt that a broad natural classification of the Terebratuloidea is achieved by division of the order into three superfamilies (fig. 9). The early radiation of the terebratuloids mainly in the Silurian and Devonian includes forms with centronelliform loops or specialized derivatives therefrom. These forms, with the exception of genera ancestral to later successful stocks are placed in the Centronellacea.

Short looped forms derived from *Craniaena* or closely related genera form a very compact and persistent group known as the Terebratulacea. Paedomorphosis is believed to have been an important feature of terebratulacean evolution throughout its course but especially in the Upper Paleozoic and Lower Mesozoic. During this time incipient long looped forms developed from paedomorphically centronelliform looped genera. A possibility is recognized that paedomorphic terebratulaceans may have been ancestral to some or all terebratellaceans though this is considered doubtful. Skipping and renewed paedomorphosis effected this group between the Permian and the Cretaceous, with skipping appearing before paedomorphosis.

Long looped forms derived from *Cryptonella* constitute the Paleozoic family Cryptonellidae and are considered ancestral to the Mesozoic Zeilleriidae. The latter group is considered to have given rise by paedomorphosis and minor structural changes in the loop to the Mesozoic and later terebratellaceans, during the Triassic. This entire group of long looped forms is considered to constitute the superfamily Terebratellacea. Paedomorphosis is believed to be an extremely important factor in the evolution of Cenozoic and Recent genera of this group.

In order to test the validity of the relationships suggested in the Terebratellacea a comprehensive study of the loops and loop ontogenies of all Triassic genera will have to be undertaken.

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EVOLUTION IN THE SUBHOLOSTEAN FISHES

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INTRODUCTION

The origin of a higher category involves a structural and functional transition from one adaptive zone to another. During the transition, the character complexes undergoing change do not necessarily evolve at the same rate or at the same time. The evidence for this conclusion is mostly provided by a few examples in the vertebrate fossil record. Actually, the great majority of families, orders and classes of invertebrates, and most higher categories of vertebrates, possess all or most of their distinguishing characters at the time of their first known appearance in the record.

Transitional forms frequently combine primitive and advanced characters in such a way that allocation of these forms to an ancestral or a descendent category becomes more or less arbitrary. De Beer (1954), in a recent discussion of the nature of the transition phase, points out that the shift from one major group to another involves animals that possess a mosaic of nearly static ancestral and evolving descendent characters. Mosaic evolution, as he terms it, is thus characteristic of the transition phase.

An important consideration in the concept of mosaic evolution is the manner in which morphologic change occurs. De Beer (*ibid.*, p. 169) states that "owing to the genetic make-up and the internal conditions of development in an organism, there may be only a limited number of changes possible, and it is legitimate to think that changes restricted to single organ-systems are more likely to have occurred than progressive changes affecting the entire organism. It is further probable that changes restricted to single organ-systems at a time were more viable and less likely to succumb to selection."

The purpose of this paper is to consider some aspects of mosaic evolution in a group of extinct ray-finned fishes called the subholosteans by a study of associations between pairs of generic characters tested by the chi square method. This approach is in a sense tentative and exploratory for reasons which will be evident later. Other groups of fossil organisms showing greater taxonomic diversification, particularly among the invertebrates, should permit more thorough statistical treatment and hence more positive conclusions in relation to association.

The subholosteans show varying degrees of advancement, from a primitive level, represented by the paleoniscoid fishes, toward a higher level, exemplified by the holostean fishes. The subholosteans, as well as the examples of mosaic evolution cited by De Beer, indicate that the transition changes do not involve single organ-systems but rather functionally integrated structural complexes that may involve components of a number of organ systems (as De Beer implies elsewhere in his paper). For instance, modifications in the feeding mechanism nearly always include changes in the skull, in the jaw musculature, and to some extent in the nerve supply and the circulation.

There is also good reason for believing that changes may occur in different parts of the body more or less simultaneously but independently of each other. All alterations must, of course, be integrated in respect to the entire organism, but this does not necessarily involve close functional interdependence of all the complexes undergoing change at any particular time.

TABLE 1

Palaeoniscoid condition	Holostean condition
1. Postrostral bone present in snout separating nasal bones	Postrostral bone absent, nasals may or may not be in contact
2. Antorbital absent, nasals forming anterior border of orbit	Antorbital present, nasals excluded from orbit
3. Maxillary fixed, in articulation with preopercular and infraorbital bones	Maxillary free posteriorly, articulating only with snout
4. Coronoid process on mandible absent	Coronoid process present
5. Suspensorium oblique*	Suspensorium vertical or nearly vertical
6. Interopercular bone absent	Interopercular bone present
7. Pectoral fin rays usually completely segmented transversely*	Pectoral fin rays only distally segmented transversely
8. Pelvic fin rays usually completely segmented transversely*	Pelvic fin rays only distally segmented transversely
9. Dorsal and anal fin rays completely segmented transversely	Dorsal and anal fin rays partly segmented transversely
10. Rays of unpaired fins more numerous than radials*	Rays of unpaired fins equal to number of radials
11. Axial lobe of caudal fin unreduced, tail heterocercal	Axial lobe of caudal fin partly or completely reduced, tail hemiheterocercal or homocercal

* Exceptions are discussed below.

Most students of fish evolution now agree that the classical subdivision of the ray-finned fishes or Actinopterygii into the Chrondrostei, Holostei, and Teleostei (Superorders of Romer, 1945) does not reflect the true phylogenetic history. Paleontological evidence accumulated during the last three decades indicates that these categories should be regarded as broad evolutionary grades. Although the fossil record is not yet sufficiently complete to work out ancestral-descendant lines in detail, it is probable that various chondrostean lines independently attained the holostean level. The phylogenies of the major groups of teleosts have not been carefully analyzed, but there is at least a suspicion of polyphyletic origin from the holostean complex.

The Subholostei (given ordinal rank by Romer, 1945) are an unnatural assemblage of advanced chondrosteans, grouped together because they possess various combinations of retained palaeoniscoid characters and definitive, or nearly definitive, holostean characters. In a few subholostean families, such as the Dictyopygidae, the included genera may be arranged to show a gradation in morphologic pattern for some character complexes from a typical palaeoniscoid condition to a holostean one.

Knowledge of the known subholostean genera is by no means uniform, mainly because of differences in the mode of preservation. The relatively few specimens preserved in a nearly uncrushed state will, of course, yield more information on the detailed structure of the braincase, the visceral skeleton and the axial skeleton than those specimens compressed to nearly paper thinness. The characters in the dermal skull and fins which are considered here can be observed in most or all of the genera regardless of the nature of preservation, and they are among the ones generally used in diagnosing the subholostean genera. The primitive (palaeoniscoid) and the advanced (holostean) condition for each character is listed in table 1 (see also figure 1).

The postrostral is a median bone capping the snout and separating the nasal bones in the palaeoniscoids. This element disappeared in most holosteans, but a more ventral element, generally called the

rostral remained. The nasal bone forms the anterior border of the orbit in all but a few palaeoniscoids, where it is partly or completely separated from the orbit by an additional bone called the antorbital. The term "antorbital" is used here to indicate the presence of one or more elements in the subholosteans and holosteans which exclude the nasals from the orbital rim. In the holosteans the antorbital complex is usually associated with a relative increase in the length of the skull in front of the orbit.

The maxillary bone is firmly fixed to the cheek in the palaeoniscoids and most subholosteans. In a few subholostean genera it is separated from the preopercular bone and from association with the infraorbital elements. This is the situation in the holosteans, where the maxillary articulates only with the front of the skull. The mandible of the palaeoniscoids characteristically has a straight or slightly concave upper border. In several genera of palaeoniscoids, in a greater number of subholosteans, and in the holosteans, the posterior portion of the upper mandibular margin is elevated into a broad process that serves for the insertion of part of the adductor mandibulae muscle.

The suspensorium is a term applied to those elements of the visceral skeleton directly concerned with the suspension of the lower jaw. The hyomandibular in particular is involved, and its backwardly directed position in most of the paleoniscoids is clearly indicated by the characteristic shape of the overlying preopercular bone. The occurrence of a nearly vertical suspensorium in some Devonian

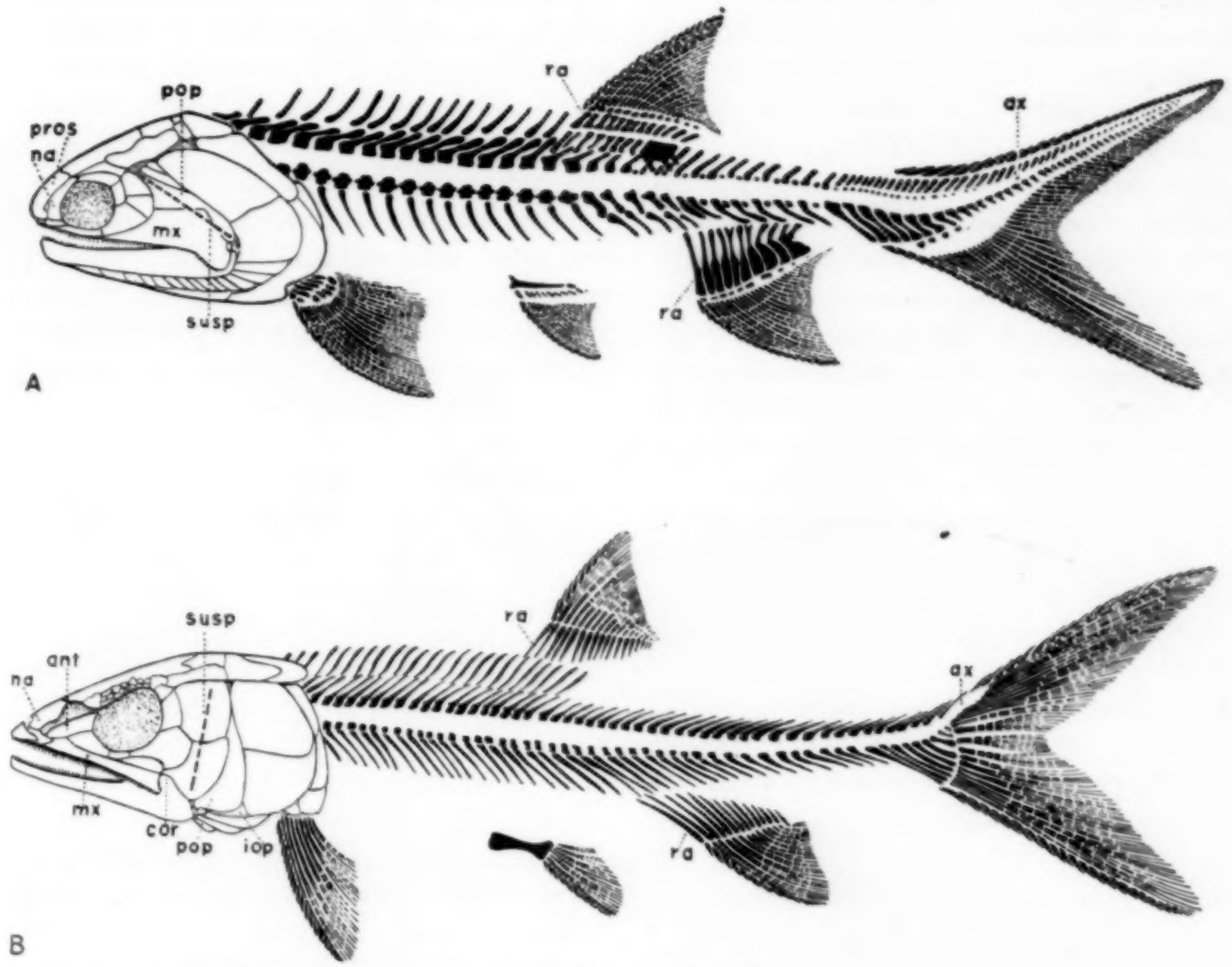

FIG. 1. Comparison of the primitive and advanced conditions.

A. The Triassic palaeoniscoid *Glaucolepis* (after Nielsen). B. The mainly Jurassic holostean *Caturus* (modified after Woodward and Rayner), ant, antorbital; ax, axial lobe; cor, coronoid process; iop, interopercular; mx, maxillary; na, nasal; pop, preopercular; pros, postrostral; rad, radial; susp, suspensorium angle.

and later palaeoniscoids indicates, however, that this orientation was about as ancient as the oblique one. The degree of obliquity was undoubtedly of adaptive significance, presumably in association with a variety of jaw mechanisms, from the Devonian palaeoniscoids until the rise of the holosteans in the late Permian and Triassic. The point here is that the common palaeoniscoid condition of oblique suspensorium associated with a characteristic pattern of the cheek bones was eliminated during the subholostean-holostean transition.

The interopercular bone apparently represents a modified and reduced first branchiostegal ray that became associated with the anteroventral corner of the subopercular. Brough (1936) believes that this modification occurred in association with the forward swing of the suspensorium and the relative reduction in the size of the jaws. It is present in some subholosteans and all holosteans except in the problematical pyncodonts.

The transverse segmentation of the individual rays of the paired fins as well as those of the dorsal and anal fins presents a problem somewhat similar to that of the suspensorium. A few paleoniscoid fishes have unsegmented rays, others have partly segmented rays, while many have completely segmented rays. The same variation is found among the subholosteans, but the greater proportion of genera show the partly segmented condition. There was, however, a general trend from complete or nearly complete segmentation toward partial segmentation during the palaeoniscoid-holostean transition. This seems evident in spite of the exceptions noted in the palaeoniscoids and subholosteans.

In all but a few palaeoniscoids, the rays of the dorsal and anal fins are more numerous than the endoskeletal fin supports or radials (baseosts). This condition, which is difficult to explain on any functional basis, may have been inherited from the as yet unknown pre-paleoniscoid stock. Two Carboniferous palaeoniscoid groups show a reduction in the number of lepidotrichia, as well as about half of the known subholostean genera in which this character can be observed. The fin rays are usually equal in number to the radials in the holosteans (see fig. 2).

The heterocercal caudal fin, in which the body axis extends to the tip of the upper lobe, is characteristic of the palaeoniscoids. The strongly heterocercal condition persisted in some of the sub-

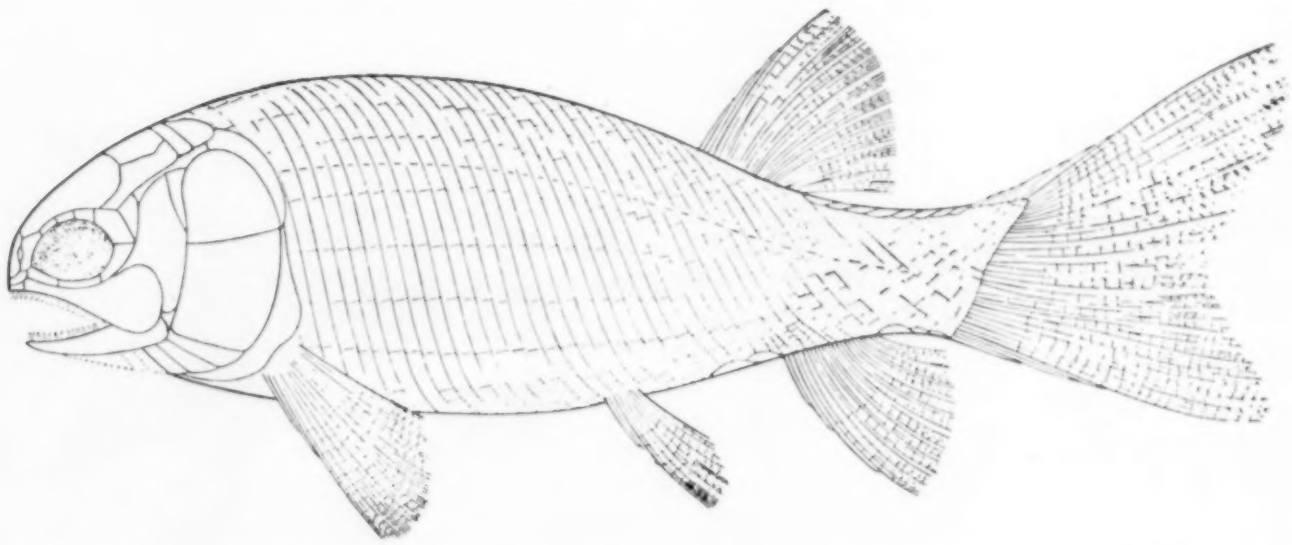

FIG. 2. *Mendocinia brevis* Bordas, a subholostean belonging to the family Perleididae. Primitive characters are: postrostral bone present, antorbital bone absent, maxillary fixed posteriorly, coronoid process on mandible absent, interopercular bone absent. Advanced characters are: suspensorium vertical, rays of all fins partly segmented transversely (see text), rays of unpaired fins equal in number to radial supports, axial lobe of caudal fin reduced.

holosteans, but more commonly there was reduction in the length of the axis, approaching, in some forms, a homocercal tail externally. It has been demonstrated by Grove and Newell (1936) that lateral undulation of the heterocercal tail elevates the posterior end of the fish, an effect that was partly counteracted by the low insertion angle of the wide-based pectoral fins which raised the front end of the body. With the development of the air bladder as a hydrostatic organ; the pectoral and caudal fins were largely relieved of the necessity of providing a lifting force to overcome the high specific gravity of the body. The caudal fin was presumably then modified into the homocercal type, which is the most efficient form for purely forward propulsion. The pectoral fins became relatively short-based with a high insertion angle, making them capable of a wide range of movement, mostly associated with stopping and steering.

Method of Analysis

The concept that characters in organisms may be associated in pairs was apparently first studied by Sinnot and Bailey (1914), who showed that in angiosperms, the presence of stipules is associated with the number of vascular bundles entering the leaf from the node. Recently, Sporne (1948, 1949, 1954) has considered associations of various floral and vegetative characters in the dicotyledons, and Stebbins (1951) floral and fruit characters in the angiosperms. Olson (1953 and earlier), employing an entirely different technique, is investigating the intercorrelation of character complexes in fossil and recent amphibians.

Associations between two sorts of characters have been investigated in the subholosteans by the chi square method. For those characters which are either present or absent (intermediate stages unknown) only two categories can be considered: primitive and advanced. For the others, which show degrees of evolutionary change from primitive to advanced, a number of more or less arbitrary categories may be designated. Because of the small number of subholostean families (13), and because of variation in character expression within many of the families, the analysis has been carried out at the generic level. Even at this level the total frequency (47) is lower than might be desired for uniformly accurate results. Actually it is nearly always below 47 for most of the associations tested since the nature of a particular character frequently cannot be determined in one or more genera.

The problem of low frequencies has usually made it necessary to combine categories, and to reduce the number of cells to a 2×2 contingency table. In the case of characters involving degrees of advancement, this has meant considering only the primitive and the advanced conditions, rather than these plus one or more intermediate states. Simpson and Roe (1939) point out, however, that if the cells in a 2×2 table have frequencies of 4 or less, it is still possible to estimate P from χ^2 with considerable accuracy by using the Yates correction.

Fifty-five character combinations have been tested for association (table 2). Of these only eleven are significantly associated ($P < .05$). Because of the low total frequency, and the fact that the adjusted χ^2 always tends to underestimate significance, the possibility must be considered that combinations bordering on significance might fall into this category if the frequencies were higher. The borderline range is considered to be $P = .10$ to .05, and four combinations are in this range. There is, of course, a high probability that several combinations, by chance, would fall into this range, but the possibility of true association cannot be disproved.

Interpretation of Results

The relatively high number of character pairs in the subholosteans that show no association contrasts sharply to the situation in the higher plants where most

TABLE 2. *The degree of association (p values) between the primitive and the advanced conditions for the characters studied. The significant associations are indicated by an *, and the border-line associations by a +.*

	Body axis in caudal fin reduced	Dorsal and anal fin rays > or = radials	Transverse segmentation of dorsal and anal fins	Transverse segmentation of pelvic fin	Transverse segmentation of pectoral fin	Interopercular present or absent	Suspensorium inclined or vertical	Coronoid process present or absent	Maxillary free or attached	Antorbital present or absent
Postrostral present or absent	.30–.50	+ .05–.10	+ .05–.10	.30–.50	.80–.90	* <.01	.20–.30	* <.01	.30–.50	.90–.95
Antorbital present or absent	.50–.70	.30–.50	.50–.70	.50–.70	.30–.50	.10–.20	.90–.95	.30–.50	.20–.30	
Maxillary attached or free	.10–.20	+ .05–.10	.10–.20	.70–.80	.70–.80	* <.01	* .02–.05	* <.01		
Coronoid process present or absent	.30–.50	+ .05–.10	.10–.20	.50–.70	.20–.30	* <.01	* .02–.05			
Suspensorium inclined or vertical	* <.01	* <.01	.10–.20	.30–.50	.30–.50	.20–.30				
Interopercular present or absent	.20–.30	.10–.20	.20–.30	.80–.90	.98–.99					
Transverse segmentation of pectoral fin	.80–.90	.10–.20	.20–.30	* <.01						
Transverse segmentation of pelvic fin	.10–.20	.30–.50	.10–.20							
Transverse segmentation of dorsal and anal fins	.30–.50	* <.01								
Dorsal and anal fin rays > or = radials	.30–.50									

of the character pairs are significantly associated. This difference cannot be explained entirely on the basis of the low subholostean frequencies, and it is doubtful if frequencies equal in magnitude to those for the plans studies of Sporne and Stebbins would appreciably alter the picture. There are, perhaps, two principal reasons for the relatively few known associations in the subholosteans: (1) absence of genetic, ontogenetic, or close functional interrelationship between the isolated characters tested, (2) absence of factual data which would make it possible to analyze in detail completely integrated parts of the body.

Stebbins (1951) has confined his attention in the angiosperms to floral and reproductive characters, with the exception of the woody habit. Most of these are closely associated morphologically and, as his analysis indicates, also functionally and phylogenetically. Sporne (1954), in addition, considers certain vegetative characters, and the high number of significant associations must reflect the same integrating factors. In the case of the subholosteans, if the data were complete enough to permit, for instance, analysis of a number of skull characters, endocranial and visceral as well as dermal, the number of significant associations would undoubtedly be greater.

The discernible changes that occurred during the chondrostean-holostean transition involved the feeding mechanism and the locomotor mechanism. Although locomotion and body form must be broadly integrated with the feeding mechanism, it is evident from this study that the skull could be altered without any obvious related changes in the rest of the body. It is further apparent that changes in the form of the paired and unpaired fins were not necessarily correlated with each other.

The character associations in the subholosteans appear to be of two sorts: (1) those involved in close functional interrelationship, and in some cases probably resulting from the same morphogenetic factors, (2) those involving more or less isolated characters that appear to be only broadly related functionally. Although the Chi square method has tested the reality of these associations, regardless of which sort is represented, it cannot eliminate the possibility of pseudoassociation (the joint association of two characters with a third). In several of the subholostean associations discussed below additional testing to detect pseudo-association is not possible because the other structures which may have been involved are known in only a few genera.

The total functional organism, at all levels of its organization, may be regarded as an inconceivably complicated network of associations. Since it would be impossible to test all the possible associations, it is desirable to consider groups of characters that appear to be functionally or morphologically interrelated. Character associations frequently may be inferred without testing, but they may also be obscure, and involve seemingly distantly integrated parts of the organism.

The associations that have been worked out for the subholosteans include three sorts of character combinations: (1) skull character with skull character, (2) skull character with fin character, (3) fin character with fin character. Allowing for the possibility of pseudoassociation, the first and third presumably had a functional basis, while the second may, or may not have represented a functional interrelationship. In discussing these combinations, the positive associations between advanced characters will be stressed, but it must be emphasized that there is also a positive association between the same characters in their primitive condition.

The absence of a postrostral bone is positively associated with the presence of the interopercular bone and with the elevation of the coronoid process on the mandible. In other words, the interopercular and the coronoid were more likely to be developed when the postrostral was absent. The loss of the postrostral was perhaps related to a narrowing of the

ethmoid region of the neurocranium, which, in turn, may have been associated in some way with a reduction in the relative size of the olfactory bulbs (these possible associations unfortunately cannot be tested). The rise of the coronoid process, and perhaps the modification of the first branchiostegal ray into the interopercular, were related to modifications in the jaw mechanism. The only apparent explanation for these associations is that the narrowing of the ethmoid region occurred at about the same time as certain changes in the jaw elements. There is also the remote possibility that some sort of an association existed between the structures involved in the water sampling mechanism (the olfactory bulbs) and in the jaw mechanism.

The associations between the jaw and cheek characters have a more obvious functional basis, and the changes involved must have increased the efficiency of the jaw mechanism. There are positive associations between the presence of the interopercular and the free maxillary, between the free maxillary and the coronoid process, between the coronoid process and the interopercular, between the free maxillary and the vertical suspensorium, and between the coronoid process and the vertical suspensorium. In view of Brough's (1936) conclusion on the origin of the interopercular, mentioned above, it is of interest that there is no significant association between the suspensorium and the interopercular.

The freeing of the posterior end of the maxillary enabled it to swing forward as the mandible was adducted. In this manner the gape was widened and the mouth cavity extended forward. The vertical suspensorium probably provided a more effective structural distribution of the forces involved in jaw movement, and it also occasioned some reorientation of the subdivisions of the adductor mandibulae muscle which arises from most of the palate. The elevation of the coronoid process altered the angle of application of at least part of the adductor mandibulae, presumably increasing the efficiency of this muscle in closing the mouth. The opening of the cheek area, primarily because of the separation of the preopercular and the maxillary, together with the vertical suspensorium, perhaps permitted an increase in the relative size and hence the power of the adductor mandibulae. How the interopercular fits into this picture is not entirely clear, although its development is clearly related to the jaw and other cheek modifications.

There are three significant associations of skull characters with fin characters, and three bordering on significance. Unless pseudoassociations were involved here, these may be examples of broadly integrated primitive characters occurring together and evolving at about the same rate into the advanced condition. For instance, the inclined suspensorium is more likely to occur with a heterocercal tail, which is the common palaeoniscoid condition, while the vertical suspensorium is more frequently present with the reduced heterocercal tail, the typical holostean condition. There is no obvious functional association, although, as pointed out above, there must have been some integration between the feeding mechanism and the mode of locomotion.

During fish locomotion the forward thrust is usually produced by lateral undulation of the body. The caudal fin also exerts a propulsive effect, and to a greater or lesser degree, depending on its size, resists transverse movement in the posterior part of the body. The form of the caudal shows no significant association with any of the other fin characters investigated. Its role during locomotion, however, is clearly related, as discussed earlier, to the angle of insertion and the width of the base of the pectoral fins. Although the association between caudal fin form and pectoral fin insertion cannot be tested because of insufficient data, it is probable that a high insertion angle occurs most frequently with the reduced heterocercal tail. There is some evidence that changes in caudal fin structure and

pectoral fin position did not always occur at the same time or at the same rate (Schaeffer, 1952).

There is a positive association between the pectoral and pelvic fins in the degree of transverse segmentation of the rays. This suggests that a single morphogenetic mechanism was involved. A decrease in the number of segments presumably increased the stiffness of these fins, thus making them more efficient for changing direction and for braking the forward motion of the body. This conclusion is supported by the observation that the narrow-based pectoral and pelvic fins, which undoubtedly could be moved in many subholosteans much as in living teleosts, occurred more frequently in forms with the reduced transverse segmentation than in those with complete transverse segmentation.

The dorsal and anal fin rays nearly always correspond closely in the amount of transverse segmentation, again suggesting a unified morphogenetic effect. Since these fins usually function as stabilizing keels, the need for equal flexibility or stiffness is fairly obvious. The positive association between incomplete ray segmentation and agreement in the number of basals and fin rays may also reflect an increase in stability for these fins.

The reason for testing a presumed character association is to determine the probability that one character is in some way related to another character more frequently than can be attributed to chance alone. In other words, as the probability of independence decreases, the reality of the association increases. There is one combination of subholostean characters out of the total of 55 tested that reaches a probability level between .95 and .99. In this case selection on the association must be almost entirely absent since the theoretical and observed frequencies are nearly similar. Thus the combination of interopercular present and pectoral fin completely segmented is the result of chance alone, and each character must be regarded as being essentially independent of the other. If all the possible character combinations in a group of organisms such as the subholosteans could be tested for association, the number showing nearly complete independence would probably be unexpectedly high. Although there is little actual evidence for this statement, it seems probable that the inconceivably complicated association network forming the integrated organism must include many structures so separated morphologically and functionally, as well as in rate of evolutionary change, that they are almost entirely independent of each other.

Conclusions

Stebbins (1951, p. 323) is of the opinion that character associations in the angiosperms "evolved through the guidance of natural selection because they represented particularly successful methods of solving problems of fertilization, seed dispersal, or both." He concludes that most of the character associations that do not actually occur in any angiosperm group are either structurally impossible or inadaptive. The associations present in the largest of the angiosperm families exist in only a few or no other families, suggesting that the smaller families have not been able to compete very successfully with the larger. Stebbins also points out (*ibid.*, p. 318) that two primitive characters may be associated, not because they are primitive, but because the association has a selective basis.

As a characteristic of mosaic evolution, it is probable that the transitional populations "try out" all the combinations of primitive and advancing characters that are genetically, ontogenetically, and functionally possible. Because of selection pressure, certain of the combinations are not successful, and some trends do not reach the new adaptive level. Similar evolutionary trends in the skull and fins were repeated a number of times in subholostean groups of diverse ancestry. These trends, involving associations of

characters that were functionally interrelated, were progressive in the sense that they approached or actually attained the holostean organizational level. The populations undergoing this structural modification inhabited a wide variety of freshwater and marine environments, but these particular trends do not seem to be related to any specific environment or mode of life. Nevertheless, the modifications were clearly adaptive since they increased the efficiency of the food-gathering and the swimming mechanisms. The principal selective factor acting on fin structure must have been the resistance of water to active motion. This may be inferred from the evidence that the typical palaeoniscoid fin form was less effective for propulsion and maneuvering than the fin pattern present in the higher bony fishes.

As Simpson (1953) has pointed out, trends typically involve more than one character. Correlated change can be produced by a variety of genetic or ontogenetic mechanisms coupled with selection. The subholostean evidence suggests that the characters concerned in a trend are mathematically associated, and that this association must frequently, but not always, reflect their functional interdependence.

There is no clear separation into primary and secondary trends for the subholostean characters investigated such as Watson (1950) has described in the skull for certain lineages of labyrinthodont amphibians. The apparent absence of subsequent or dependent trends may be a reflection of inadequate data, but it is more likely the result of nearly simultaneous change coupled with close functional integration.

The manner in which selection operates in relation to association and evolutionary trends involves the concept of the character complex. Characters that are closely related functionally can be changed primarily or secondarily by a single genetic or ontogenetic mechanism. In the living organism, however, the composition of the complex may be difficult to define, and it may include structures that are not closely associated morphologically. If positive linear selection acts on two components of such a character complex, a single trend is produced and there is true association between the parts. The trends in the subholostean jaw mechanism are probably examples of this situation. Here selection favored both the trend and the association net of an integrated complex.

When positive linear selection acts independently and essentially simultaneously on two characters that have no direct functional interrelationship, the result will be two separate trends and a true association. In this case, the association is obviously false. But in any specific example, it may be impossible to present a convincing argument for the independence of these characters. The associations of skull and fin characters in the subholosteans may be spurious, but, on the other hand, there may be broad functional integration between them, implying at least closely correlated trends, or perhaps even a single trend, and true association.

If one character is subjected to positive linear selection and the other to no selection, or to stabilizing selection, there will be no association, although the first character is involved in a trend. The absence of association in this case results from the lack of genetic and functional relationship between these characters, making it impossible for them to participate in a common trend. Since all the characters selected for the subholostean study show change, this situation is presumably not represented.

By employing Yule's coefficient of association, the intensity of association for the characters involved in the jaw mechanism has been measured. When the coefficient value is zero, the characters are completely independent, while 1 (unity) represents complete association. The positive association between the free maxillary and the coronoid process, or between their primitive counterparts, has a value

of + .99 indicating nearly complete association. For primitive and advanced associations of the other characters directly concerned with the jaw mechanism, the coefficient values also border on unity, again indicating almost complete dependence. From these results it may be reasonably concluded that the intensity of association must vary with the degree of functional interrelationship. The coefficient value also approaches unity when primitive characters occurred together that were at most distantly related functionally, but evolved at about the same rate to the advanced condition. Here the value may reflect, in part, the equality of the evolutionary rates.

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Summary

The combination of primitive (palaeoniscoid) and advanced (holostean) characters in the extinct subholostean fishes represents an example of mosaic evolution in a transitional group.

By means of the chi square method, associations of certain skull characters and fin characters were determined at the generic level. Some combinations of skull characters and of fin characters represented functional complexes probably resulting from the same genetic and ontogenetic mechanisms. The character associations within these complexes may therefore be regarded as true associations acted upon by the same selective factors. Significant associations between skull characters and fin characters were true associations providing there was some sort of functional integration, otherwise these associations must be regarded as spurious. A broad functional relationship between the feeding and locomotor mechanisms represents a possible unifying factor for these relatively isolated characters.

Similar trends in the skull and fins occurred a number of times in unrelated subholostean families that inhabited a variety of aquatic environments. The trends involved functional character complexes, and the components of the complexes were associated. Both the trends and the associations were guided by selection. During a transition period positive linear selection favors trends which are of immediate selective advantage. Thus some complexes remain primitive while others show advance, resulting in the mosaic pattern. It is probable that in transitional populations all the viable combinations of primitive and advanced characters are acted upon by selection. The populations involved in the break-through into the new adaptive zone possess the character complexes which are functionally adaptive for that zone.

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REPRODUCTIVE ORGAN POLYMORPHISM IN EARTHWORMS OF THE ORIENTAL MEGASCOLECINE GENUS *PHERETIMA* KINBERG 1867

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Absence of certain organs ordinarily essential for biparental reproduction in normally hermaphroditic oligochaetes was recorded for certain Japanese species in 1892. These defects have puzzled oligochaetologists ever since and have been responsible for considerable unresolved confusion in the taxonomy of the genus.

Evolution of secondary bisexuality from ancestral hermaphroditism was invoked to explain the situation characterizing one Burmese species (*anomala*) by Stephenson (1929) who had been told (by Pickford, *in lit.*) that there are, in a South Pacific species, "functional females" as well as "functional males." Supposed females in *anomala* very often, perhaps always, produce sperm and profusely. Supposed males, on the contrary, always have apparently normal ovaries, sometimes even an extra pair but only rarely mature sperm (Gates, 1933, p. 507), and then sparsely. An occasional individual, in several species of *Pheretima*, obviously is so circumstanced (1954, and below) that it may be able to function as a female but for existence of a male sex in earthworms no evidence has been found.

Selective inhibition, during juvenile growth, of organ development by metabolic products of gregarine (?) parasites was thought (1932, pp. 388, 497–501 and 1933, pp. 508–510, 520–521) for a time to be responsible for the "abnormalities." Effects on host earthworms of the presence of such large numbers of parasites still are to be determined but it was eventually recognized that maintenance of organ absence year after year at the same localities, in high percentages of the populations—sometimes approaching or even reaching 100, required change in the supposedly usual method of earthworm reproduction. Furthermore, the agency responsible for production of the most drastic morphological deletion may well be effective during embryonic development and thus prior to the time when parasites were thought to exert their influence.

The reproductive system of Japanese species of the genus, according to Ohfuchi (1939, p. 82) who studied the same deviations from generic norms, is "markedly sensitive to the effect of environmental factors." Differences in temperature and rainfall (*idem*, p. 116) were the only factors definitely mentioned.

Data as to deletions in the reproductive system of earthworms, from many widely scattered taxonomic contributions, have been reviewed together with unpublished accumulations. Considerable information is available for several species of *Pheretima*. Those considered below have, collectively, all of the various morphs and provide illustrations of typical stages as well as sequences in evolutionary development. The remaining species, including those of other earthworm genera and families will be considered, if circumstances permit, in a subsequent communication.

The reproductive system and reproduction in *Pheretima*.

This system normally comprises the following structures, usually in segmental pairs:

Testes. In segments x–xi, on posterior faces of septa, near ventral parietes.

Testis sacs. Closed off portions of coelomic cavities containing testes and

male funnels. Opening into seminal vesicles through apertures in posterior septa.

Seminal vesicles. Sacs, in xi–xii, on posterior faces of septa 11/12–12/13, in which sperm are matured. With testis sacs, male ducts and male terminalia constituting a system from which, so far as is known, sperm escape only through the male pores.

Sperm ducts (male deferent ducts, vasa deferentia). Opening into testis sacs through plicate funnels (male) and posteriorly into innermost portions of ducts of large, racemose "prostates."

Male terminalia. Male pores always behind clitellum, usually on xviii, occasionally on xix or xx according to species. Each pore may be in an indistinctly demarcated area of slight epidermal modification, in a small disc or tubercle retractile into a parietal invagination or on some sort of protrusible penis within an eversible copulatory chamber that reaches more or less conspicuously into the coelom. Glands, one or more, of varied structure and of unknown function, may open onto male porophores or penes or into invaginations and chambers. Follicles of more or less modified setae may open onto porophores, into invaginations or chambers. All of these associated structures, including the prostate and its muscular duct, are collectively known as male terminalia. A worm which lacks terminalia of one or both sides is mono- or bi-laterally anarsenosomphic.

Ovaries. In xiii, on posterior face of 12/13 near ventral parietes.

Oviducts. Short ducts, opening into coelomic cavity of xiii through funnels on the anterior face of 13/14, with an external aperture on xiv.

Spermathecae. For storage of sperm received from another individual during copulation. The only openings into these organs are on the surface of the body. Each has a saccular ampulla, possibly for storage of prostatic secretions and a duct with a more or less elongate diverticulum. Spermathecae always are anterior to testis segments and hence well in front of clitellum. Number varies according to species. There may be one to six pairs, pairs of pairs or pairs of groups (polythecal).

Clitellum. An annular modification of epidermis of xiv–xvi for secretion of an egg case or "cocoon." Appears at maturity and regresses after breeding season.

GM glands. These are of various kinds and presumably function in connection with reproduction. Usually associated with modified areas in the epidermis called genital markings (GM) which may be superficial, retracted more or less deeply and then in individual invaginations or in those containing spermathecal pores (also in those containing male pores).

At maturity, testes have lost their characteristic juvenile appearance. Seminal vesicles no longer have a characteristic juvenile shape and firmness but are large and of a much more delicate texture. Sperm have been passed back into testis sacs from the vesicles and are aggregated on male funnels so as to refract light with a brilliant iridescence. Male deferent ducts, in appropriate conditions, have some iridescence.

After copulation, iridescence indicates presence of sperm in the spermathecal diverticula and occasionally also in one or more pseudo-spermatophores imbedded in non-iridescent material within the ampulla. Spermatophores never were found on the surface of the body of many thousands of worms collected during the breeding season.

Cocoon deposition has not been observed in this genus but worms presumably wriggle backward out of the case and into it, as they do so, extruding first ova through the female pore and then sperm through the spermathecal pores. Development takes place within the cocoon.

After breeding, a brown granular material appears in seminal vesicles and may

be compacted into formed bodies. Vesicles become somewhat shrunken, but without returning to a juvenile condition. Testes do not, at least for some time, regain a juvenile appearance. Iridescence of male funnels and spermathecae gradually disappears but sometimes persists, in one or both sets of organs, after clitellum has become unrecognizable.

Iridescence never appears on male funnels, in testes, seminal vesicles and coagulum of testis sacs of some individuals. This conclusion, reached by the author in the late twenties (1932, pp. 308–309), after examination of material collected in the tropics throughout the single period when earthworms are active, provided the initial stimulus to study of the problems herein considered.

Absence of iridescence can mean: (1) Morphology of functional sperm has undergone some radical change. (2) Sperm no longer aggregate in such a way as to refract light. (3) Male gametes are produced elsewhere than in testes at usual sites. (4) Worms are male sterile. No evidence had been found, at the time the study of oriental forms was abruptly terminated by World War II, for the first three alternatives. Any earthworm gonad, testis or ovary, may however have potentiality of becoming hermaphroditic though maturation of sperm in ovaries has not as yet been recognized in any species of *Pheretima*.

The proper range of the genus *Pheretima* extends from Burma into Korea as well as Japan and through the Malaysian islands including New Guinea but probably not to Australia. In that area, earthworms have been collected intensively only in certain sections of Burma, China, Korea and Japan. Number of known species was thought to be 300 by Stephenson in 1930, since when many more have been erected. In the next largest earthworm genus, *Dichogaster*, there are, according to the same author, only 163 species.

All data cited or referred to, except as otherwise indicated, are from the author's studies.

Pheretima anomala Michaelsen 1907

Individuals of a morph to be called "H" (having a full hermaphroditic complement of reproductive organs) usually are lacking in collections (see note 1, table 1) but often can be secured, in limited numbers, if the search is sufficiently prolonged. Several hundred Burmese specimens have been available, mostly from Rangoon where collecting was continued through much of the period between 1923 and 1942. Highest incidence of H worms (table 1), about twenty percent, was in lots from Kengtung and Koopra which may be nearer the original home of the species than are middle and western Burma. Sperm are matured and have been found in spermathecae. Biparental reproduction ordinarily can take place, however, only after copulation with another individual of the same morph, an event rarely to be expected in many localities. Reproduction, even in this morph, frequently must be uniparental.

A second morph, to be called "R," is distinguished by absence of male terminalia as well as of all mushroom-shaped (GM) glands of xvii–xix. Male deferent ducts never acquire external openings though they may grow back, before attenuation, to any level between xv and xxxi. Sperm usually (always?) are matured (1933, p. 507) and production seems to be profuse though expulsion from the body is impossible in absence of male pores. Sperm occasionally found in spermathecae of an R individual, since copulation with another R worm or with itself would be futile, must have been received from an H individual. This can happen only infrequently because of the rarity of that morph. Reproduction in this morph also must often be uniparental.

One other morph, to be called "A," is distinguished from the H by the presence of one to four extra pairs of GM glands behind xx and by absence of the entire battery of six spermathecae. Male termi-

TABLE 1. *Polymorphism in* Pheretima anomala

Numbers of individuals of various sorts in representative collections from Burma and India

A	I	H	I_2	R	Locality	District
119			1		Heimza Basin	Tavoy
377			31	11	Kyaukmedaung to Kameik (Aug.)	Tavoy
69			8	2	Kyaukmedaung to Kameik (Oct.)	Tavoy
14		1	10	6	Loikaw	Karenni
5		21	9	67	Koopra	Karenni
6			9	1	Mala	Karenni
				148	S'nite	Karenni
2			2		Thandaung	Toungoo
9			2	5	Kalaw	Shan States
45		4			Taungyi (1932)	Shan States
32		7	3	293	Taungyi (1933)	Shan States
15		24	16	67	Kengtung	Kengtung
4			2	3	Loi Se	Mang Lun
1				1	Akyab	Akyab
1					Tiddim	Chin Hills
7					Falam	Chin Hills
1		1			Myitkyina	Myitkyina
32		5	5	63	Kokine (1932)	Hanthawaddy
+	+	+	+	+	Rangoon	Hanthawaddy
+	+				India (four localities)	

A Athecal.
I Intermediate between A and H.
H With full complement of reproductive organs.
I_2 Intermediate between H and R.
R Anarsenosomphic. GM glands and markings also lacking.

Note 1. These worms, as well as those of other Burmese species, usually were from collections made in order to learn what species were present in a locality.

Note 2. Sperm are matured in small amounts in an occasional individual of the A morph, in two of 250 adults in a 1928–32 series (1933, p. 507).

Note 3. Self fertilization has been suggested as a possible method of reproduction in one earthworm, the ocnerodrilid *Eukerria saltensis* (Beddard) 1895, but even in that species Gavrilov (1952) admits possibility of parthenogenesis is not yet ruled out.

Note 4. Originally the genus *Pheretima* was quadrithecal, with the spermathecae in viii–ix. The battery has been augmented, presumably, by addition of organs, first in vii and then in vi. From an octothecal ancestor, *anomala* would then be derived by elimination of the last pair of spermathecae, those in ix.

Note 5. Some records are available in Gates, 1933, pp. 505–506. Mention of other data is now impossible, records of later years and most of the material, of this as well as of other species having been destroyed in Burma during World War II.

Note 6. Male terminalia are in xviii in most species of *Pheretima* and other megascolecines. An ancestral stage with terminalia in xix may have preceded *anomala* in which the terminalia are in xx. The male pores were equatorial in each of the specimens of *anomala* whether pores were on xviii, xix, xx or xxi.

Note 7. The anterior pair of spermathecae was, however, lacking in one worm.

nalia are present and apparently normal (prostates large) but seminal vesicles usually are small, rudimentary or lacking and testes remain juvenile (note 2, table 1) through the reproductive season.

Reproduction, when sperm are not matured and cannot be received, as in this A, must be not only uniparental but also parthenogenetic. That presumably is also the usual method of uniparental reproduction in the H and in the R though autogamy cannot as yet be ruled out in all cases (Note 3, table 1). Parthenogenesis is assumed however to be facultative in *anomala* though morphological or environmental conditions may deny option of biparental reproduction.

Conspecificity of the three morphs is

demonstrated by the intestinal caeca, the spermathecal battery, the shape of the individual spermatheca, anatomy of GM glands as well as of male terminalia, the very structures on which generic taxonomy necessarily is based, and by "intermediates." Those demonstrating relationship between A and H differ from the A in the presence of a single normal H spermatheca and are very rare. Less than a score were found during twenty years collecting and that number includes at least one in which the spermatheca was quite rudimentary. Intermediates of this sort are referred to as of a first order or class. Usually the spermatheca is at one of the positions of an H battery, in vi–viii. Origin from an intermorph cross, such as occasionally may be possible, was suggested in a previous contribution (1954) but is ruled out in India where only the R is present. Intermediates of the first order in India must then have been of a lineage in which spermathecae had not yet been completely eliminated or else were mutants in a direction back toward H. The second alternative seems more likely in the case of the worm with one spermatheca in ix rather than in any of the H positions though even that could be a reversion to a much more remote ancestral stage (Note 4, table 1). Intermediates, indicating by characteristic structure relationships between two morphs, that cannot be in direct line of evolution from one morph to the next in the series are distinguished by the designation "pseudo-."

Intermediates of a second order (I_2) demonstrating relationship between H and R, also are of two sorts. One differs from the H by absence of GM glands (Note 5, table 1), by absence of those glands and additionally of male terminalia on one side, by absence of terminalia of both sides and of all but one, two or three of the GM glands. GM glands almost always seemed to be normally developed. Male terminalia usually appeared to be normal though a prostate gland occasionally was lacking when the duct was present (1933, pp. 505–506). Evolution, from the H into an R morph, appears to be incomplete in such worms and to be taking place by dropping out entire organs one after another or by elimination of groups of organs simultaneously.

Pseudo-intermediates of the second order have characteristic H male terminalia but in xix or xxi(rather than xx), characteristic GM glands in A locations or even in xx and then at sites of male terminalia. Mutation back to an ancestral stage more remote than that represented by the present-day H seems possible in worms with male terminalia in xix or xviii (Note 6, table 1) but those with terminalia in xxi show an additional translocation that was not involved in the ancestry of the H.

No indications were recognized of any trend toward reduction in size or elimination of useless male terminalia and of GM glands in individuals of the A morph nor of spermathecae (Note 7, table 1) in the R. On the contrary the A has more glands than the H. Both morphs appear to be stable with respect to their special characteristics.

Deviations from specific norm additional to those already mentioned have been recognized only in that morph (A) in which morphological and environmental conditions almost always seem to require parthenogenesis. Among the more important are the following: 1) Retention (occasionally), sometimes with muscularization, of a complete septum 8/9 (instead of abortion). 2) Retention throughout postembryonic life of testes in some or all of v-ix (instead of abortion), with or without induction of corresponding male funnels and some portion of their ducts. 3) Retention (occasionally) throughout life of gonads in xii (usually agonadal in juveniles as well as adults). These gonads usually are, at least in part, female. 4) Modifications of testis sacs which may be paired (instead of unpaired), vertical (instead of ventral and transverse), U-shaped, cylindrical, of other less normal sorts, or even lacking.

Evolution of A and R morphs, ordinarily denied biparental reproduction, presumably was preceeded in *anomala* by acquisition of ability to reproduce parthenogenetically. Genetic factors subsequently involved in divergence of the two derived morphs appear to be mostly inhibitory, suppressing development of spermathecae and (usually) maturation of sperm, nullifying a long-standing oligochaete abortion of embryonic gonads in all segments except x–xi and xiii, in the A; suppressing development of male terminalia and GM glands in the R. Only in the A is there evidence of action of less negative factors, the development of extra GM glands which are however probably useless in the circumstances ordinarily prevailing. Genetic determiners responsible for the special characteristics of each morph presumably are lacking in the other though coexistence of all of them in the same genotype apparently is not impossible (cf. below).

P. anomala has no close relatives in the mainland portion of Burma and its origin presumably is to be sought to the east or southeast. Unusually wide distribution throughout middle and western portions of Burma may well have resulted, in part, from transportation. There seems to be no reason for doubting that the species was but recently introduced into the four widely separated localities where it has been found in India. A uniparental method of reproduction appears to be advantageous in the establishment of new colonies after such transportation.

Pheretima alexandri (Beddard) 1900

The normal H morph of this species at maturity has large, soft, shapeless seminal vesicles and slender spermathecal ducts. Reproduction presumably is biparental ordinarily though detailed records no longer can be cited (Note 5, table 1) as to maturation and reception of sperm. This morph has been recognized outside of Rangoon only in eastern portions of Burma that may be nearer the original home of the species.

Other H worms have smaller seminal vesicles, of firmer texture and more or less definitely juvenile appearance. Spermathecal ducts are thicker ectally and the bulbous portion has a marked muscular sheen. Method of reproduction cannot now be stated but little or no sperm production is anticipated. Worms of this sort are common in middle as well as western Burma and have been found in the Andaman Islands, Calcutta and Bombay undoubtedly as a result of recent transportation. Attempts to distinguish two morphs by external characteristics were unsuccessful. Spermathecal ducts were of no more practical use for routine identification because of occasional occurrence of the muscularization in slight to very slight degrees. Variation in size of seminal vesicles also was found (possibly correlated with amount of sperm produced?). Nevertheless, two sorts of H may be said to exist: One, in which sperm are matured. Another, H_p, in which no sperm are matured and in which reproduction must be parthenogenetic except possibly after copulation with a sperm producing individual.

An A morph has not been found, nor an R, but three specimens of a short series (table 2) from Kawkareik, near the Siamese border, belong to one lacking in *anomala*. This morph, to be called the "AR," not only has no spermathecae but also no male terminalia. Testes are juvenile and seminal vesicles are rudimen-

TABLE 2. *Polymorphism in* Pheretima alexandri

Numbers of individuals of various sorts in representative collections from three districts of eastern Burma

H	I_4	AR	Locality	District
2		3	Kawkareik	Amherst
325	32		Kengtung	Kengtung
69	1		Loikaw	Karenni

I_4 Intermediate between H and AR.
AR Athecal and anarsenosomphic.
H With full complement of reproductive organs. H_p (parthenogenetic) not here distinguished.

tary in worms with maximal clitellar tumescence. Reproduction, in the AR, must be parthenogenetic even if ova still are capable of fertilization.

All intermediates (33) for which records still are available were from eastern Burma near the Siamese boundary. A male porophore, more or less aberrant but enabling specific identification, was present in ten of them on one side of the body only. One prostate and its duct was present in one worm, one short duct without prostate gland in four, ducts as well as glands lacking in the others. All spermathecae were present and at H sites (in vi–ix in this species) in 16 worms but some were lacking in each of the others; in ix (15 worms), in vi and ix (1), in viii and ix (1). The spermathecal diverticulum was elongate but without differentiation into stalk and seminal chamber, quite rudimentary or lacking. Additional deviations from specific norm shown by these intermediates of a fourth order (I_4) were; slight extension of clitellum onto xvii (9), oviducts opening individually to the exterior instead of uniting mesially (20). Sperm had been matured in some of these defective worms and terminal portions (here in xii-xiii) of incomplete male deferent ducts were distended by gametes that could not be discharged because of absence of male pores. As sperm were not found in adiverticulate spermathecae, or even in abnormal diverticula, reproduction presumably would have to be uniparental. Autogamy seems unlikely. Evolution to an AR morph presumably still is incomplete in these worms.

Evolution of an AR morph in *alexandri*, according to the evidence now available, takes place only from an H by concurrent modification and, eventually, elimination of all spermathecae as well as all of the male terminalia. Sperm producing intermediates presumably have been derived only from a sperm producing H but male-sterile intermediates may have been derived from a male fertile or a male sterile H. Parthenogenesis obviously may precede suppression of sperm maturation in *alexandri* (also *cf.* R morph of *anomala*), and may even be possible in the supposedly biparental H morph. Concurrent acquisition of parthenogenesis and male sterility also seems to be possible. If, however, sperm development is inhibited in an individual prior to establishment of parthenogenesis, reproduction would be impossible for the copulatory partner (except of course after subsequent copulation with another and male fertile worm) though still possible for the male sterile worm if the partner had been male fertile.

Pheretima campanulata (Rosa) 1890

The H morph of this species has large seminal vesicles and prostates. Sperm are matured and their presence in seminal chambers of spermathecae shows that copulation takes place. Biparental reproduction presumably can be expected. This morph is the only one that has been found in India and the Andaman Islands where this species has been introduced, as well as in various localities of the middle and western Burma. Incidence of H was however only *ca.* 43% in one Burmese series (Toungoo district) and in collections from Bhamo, Kengtung and Taungyi (eastern Burma) fell to *ca.* 5%, 5% and 2%, respectively.

An A morph, as in *anomala*, is characterized by absence of a battery of six spermathecae. Seminal vesicles always are present but are small, usually of juvenile size and shape. Testes usually are juvenile in adults. No evidence of sperm maturation has been recognized. Reproduction must be uniparental in absence of spermathecae and, if sperm are not produced, parthenogenetic. Additional deviations from specific norm always are recognizable: Penial setae lacking in copulatory chambers. Clitellar setae irregularly furrowed. Penes usually with 3–5 small genital markings (instead of 2) at distal end. External genital markings and associated glands almost always lacking (as occasionally in the H).

An R morph never was recognized but a few individuals of an AR (table 3) were secured, records for nine of which still are available. Testes are juvenile and seminal vesicles are rudimentary, as in the *alexandri* AR, in fully clitellate individuals. Reproduction must be parthenogenetic.

One morph not yet recognized in any other species (of any earthworm genus) was represented by at least two mature specimens. Incidence in the short series (table 3) in which it was recognized, 25%. Each of these "ARZ" worms was athecal, anarsenosomphic, and also lacked genital markings, GM glands, seminal vesicles, testis sacs, male funnels, male deferent ducts and *testes*. As development of vesicles and funnels (with their deferent ducts) is thought to be induced by male gonads, absence of those organs indicates early ontogenetic abortion of testes.

Intermediates usually are rare in this species and for several hundred of the A morph in Rangoon collections prior to 1932 only two had been obtained. They are however more frequent in some localities and incidence in a series from the Pegu Yomas was as high as *ca.* 9%. Intermediates in *campanulata* again are of several sorts. The most common apparently are those between H and A.

TABLE 3. *Polymorphism in* Pheretima campanulata

Numbers of individuals of various sorts in representative collections from fourteen districts of Burma

A	I	H	AR	ARZ	Locality	District
2		2			Sittang	Thaton
18	1	7			Kyaikto	
1		14	2		Thaton	
1	3	2		2	Duyinzeik	
4		22			Kumingyaung	
3		3			Taungzun	
1		11			Dam-site	Insein
5	1	19			Bassein	Bassein
		3	1		Myohaung	Myaungmya
8		5			Pantanaw	Maubin
1		4			Danubyu	Maubin
2	10		2		Laboo	Prome
62	13	59	4		Pegu Yomas	Toungoo
4	2	16			Shwegyin	
13	1	17			Kyaukkyi	
16	3	2			Pa Taw Lo	
3	1	16			Pauk-taw-gwin	Salween
	1	76			Mewaing	
		11			Paletwa	Arakan Hill Tracts
5					Katha	Katha
	3				Wuntho	
87	71	3			Taungyi	Shan States
150	x	8			Bhamo (1932)	Bhamo
19	x				Bhamo (1954)	
42	x	5			Kengtung	Kengtung
+	+	+			Rangoon	Hanthawaddy

A I H AR As in previous tables.

ARZ Athecal, anarsenosomphic, testes aborted, testis sacs, male deferent ducts as well as their funnels and seminal vesicles lacking.

x At least 18 listed under A belong here.

Note 1. Five worms, otherwise like intermediates of the first order, lack one prostate, the prostatic duct present but rudimentary in two of them.

Note 2. Whether heavily parasitized worms are more likely to be secured by the average collector is as yet unknown.

These (I) differ from the A in having a more or less rudimentary spermatheca at one or more of the positions of the H battery. In 102 worms, where there should have been 612 spermathecae, only 166 were present. Of the three levels, spermathecae were retained most frequently in viii rather than vii or ix (1933, p. 516). The parietal portion normally developed in several spermathecae (with 2 stalked glands opening as usual into the parietal invagination), a diverticulum normally developed in several other spermathecae, enabled specific identification of these intermediates as well as placement of the A morph (with the same male terminalia) in *campanulata*. Presence of sperm in a normal diverticulum, even though the main axis was rudimentary, showed that male sterile worms do sometimes copulate with a sperm producing individual. As a result the male sterile, sperm receiving individual is in a position to reproduce in the usual biparental manner of an H morph (unless of course parthenogenesis had become obligatory) though the fertile partner must rely on parthenogenesis (if that is possible) to reproduce itself!

A less common sort of intermediate (I_5) differs from the A in degree of development of male terminalia. Conditions briefly characterized below were found.[1]

1) Prostates in xvi–xx, prostatic ducts 5–7 mm long. Copulatory chambers, associated stalked glands and penes present. (Normal for A.)
2) Prostates in xvii–xix.
3) Prostates confined to xviii.
4) Prostates quite juvenile or rudimentary.
5) Prostates absent. Ducts 5–7 mm long.
6) Ducts much shortened.
7) Ducts lacking.
8) Copulatory chambers rudimentary, glands and penes lacking.
9) Copulatory chambers absent but sites of apertures indicated by gaps in setal circles and epidermal irregularities. Except for these parietal vestiges indistinguishable from the AR.

These I_5 individuals show that the AR in *campanulata* may be preceded by an A.

Maturation of sperm in A individuals (probably also in first order intermediates) must be at least as rare as in the *anomala* A. Parthenogenesis must have been a prerequisite to evolution of secondary morphs just as in *anomala*.

Evolutionary trends in *campanulata*, judging from conditions shown by the intermediates that have been available, are in one direction only, toward a more advanced evolutionary morph. The usual sequence now appears to be: H → (I) → A → (I_5) → AR → ARZ, though one short circuit occasionally may be possible (Note 1, table 3). The end result of the evolutionary processes apparently under way in *campanulata* will then eventually be ARZ, an emasculated morph in which no vestiges of male organs and of spermathecae are recognizable in adults. Nevertheless, testes may not have been finally eliminated but only aborted in early ontogeny.

Smaller evolutionary steps in reduction of spermathecal battery and of male terminalia, than in *anomala*, are suggested by intermediates of *campanulata* and *alexandri*. The difference, however, may be largely one of timing of initial effect of modifying factors or of inhibiting determiners in ontogeny. Even sterilization of germinal tissues in the testes (to be considered later) may proceed gradually. In any case, genetic determiners here involved must, as in *alexandri*, *levis*, etc., be capable of operating or being inhibited unilaterally, and additionally in case of the spermathecal battery, differently in each of the adjacent spermathecal segments (two to six in other species).

[1] This is a condensed outline. Deviations from normal may be restricted to one side of the body, or each side may be differently affected.

Parthenogenetic individuals of *campanulata,* as well as of several other Burmese species, very frequently were infested by gregarine parasites rarely if ever present in such masses in sperm producing individuals (Note 2, table 3). Coelomic cavities of v–vi may be crammed with cysts, the coelomic face of parietes and/or gut covered with cysts in several to many segments, cysts numerous in coelom of each segment all the way back to the anal region. Seminal vesicles sometimes distended to sexual size by cysts. Less frequently similar cysts in or on larger blood vessels (including hearts) and the spermathecae, larger cysts of unknown nature within the parietes and in the nerve cord. Availability of metabolic products no longer required for spermatogenesis, may permit, in a monsoon climate where there is but a single breeding season each year, massive infestations without much harm to the host.

Two taxa closely related to *campanulata* will next be considered.

Pheretima meridiana Gates 1932

This taxon always has been distinguishable from *campanulata* (H) as follows; copulatory chambers slightly larger and with smoother dorsal surface, spermathecal invaginations slightly larger and just reaching into coelomic cavities, penis with bifid (rather than trifid) tip and having thereon only one genital marking (instead of 2), penial setae lacking, seminal vesicles small and apparently juvenile. Differences are of about the same sort, except for organ defects, as distinguish the A of *campanulata* from the H. *P. meridiana* has been transported recently to the Bahamas and has been found west of the Irrawaddy-Sittang axis only in the Pegu Yomas and in Myitkyina district. Its range coincides, in part at least, with that of *campanulata.*

An R morph certainly would be difficult (impossible?) to distinguish from that of *campanulata* and if it exists has not been recognized. Only one individual of an A morph has been found.

Male sterility, or but sparse sperm production, and parthenogenesis is anticipated. In that event, *meridiana* may prove to belong to a geographical race of *campanulata,* the apparent overlapping of ranges now permitted by reproductive isolation.

Pheretima houlleti (Perrier) 1872

This taxon, except for a rather general smaller size of soma and a more anterior location of the first dorsal pore, differs from the *campanulata* H in characteristics of about the same sort as distinguished *meridiana.* The range, probably considerably extended by recent transportation, coincides in part with those of *campanulata* and *meridiana.*

Seminal vesicles of adults apparently always have a shape, size and appearance that in *campanulata, alexandri,* etc., would denote retention in a juvenile condition. Sperm have been found in spermathecae but production may well be sparse usually. Parthenogenesis is anticipated. Secondary morphs have not been recognized.

If *houlleti* and *meridiana* prove to be conspecific with *campanulata,* for each of the three H morphs sufficiently distinctive to have been treated as a species, a complete set of four secondary morphs may be possible (fig. 1). Not all of these will be recognizable anatomically.

Pheretima levis (Goto & Hatai) 1899

No H morph has been represented in American material from New York and New Jersey. Two of the available worms were R, one was AR and five were intermediates of a third order enabling identification of the AR (Gates, 1954). Eighteen other AR worms from the same localities may have been of this species but just as well could be of two other Japanese exotics. Seminal vesicles were small. Additional deviation from generic norm, abortion of hearts of x. No evidence of sperm maturation or reception was recognized in any of the worms which all had a fully matured clitellum.

An H morph, even in the home of the

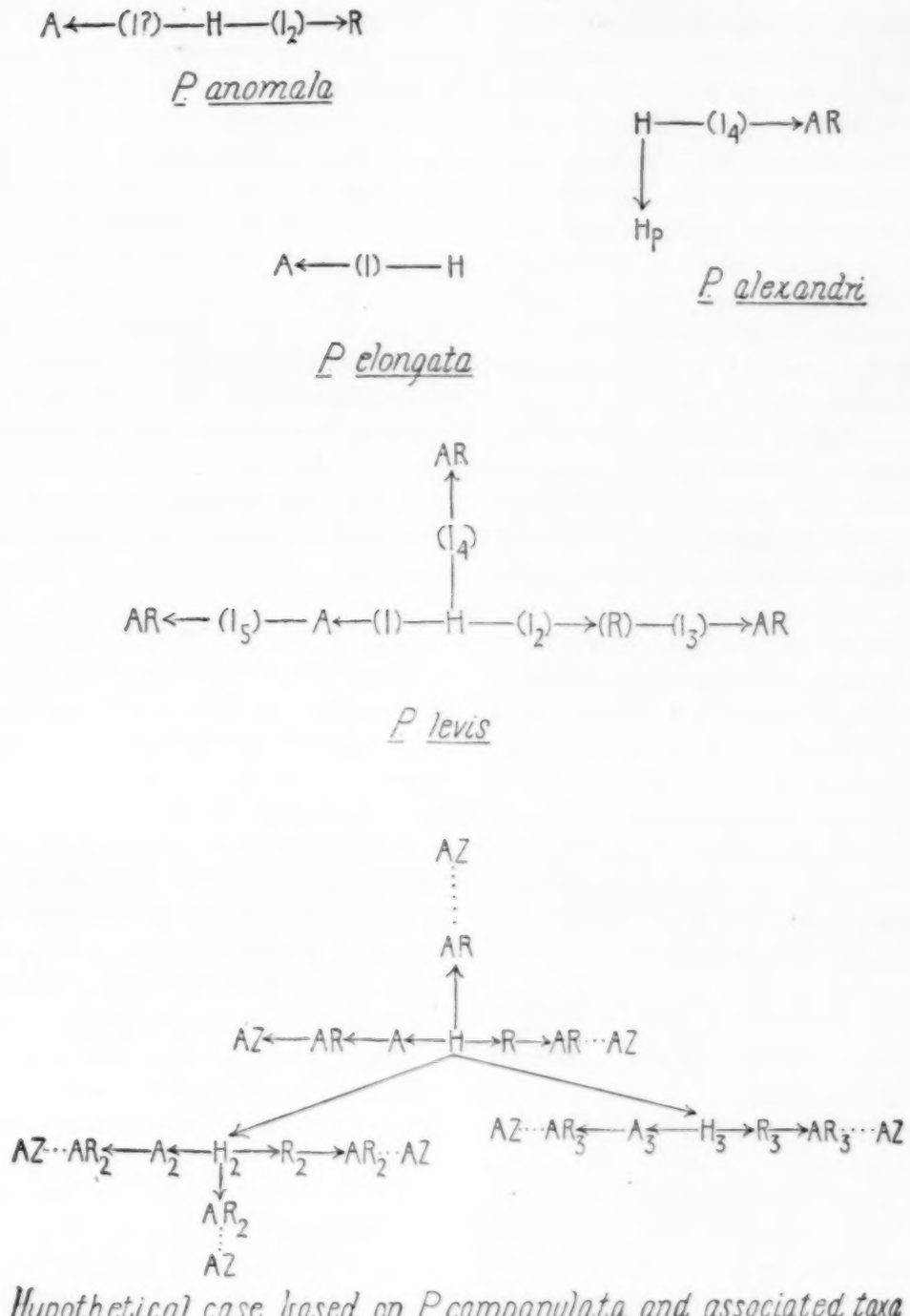

FIG. 1. Reproductive organ morphs in several species of *Pheretima*. (Note. The designation of the terminal morphs in *campanulata* should be ARZ.)

species, would seem to be very rare, according to the data of Ohfuchi (1938, 1939), as it was represented only by 40 (1.3%) and 33 specimens respectively in two series of 2875 and 3220 worms (see table 4). Male terminalia were lacking in 2157 (75%) and 2272 specimens respectively of those same two series, in which spermathecae were absent in 1653 (57%) and 2024, respectively. An AR morph was common, being represented in the two series by 1256 and 1414 individuals, respectively.

Variation in spermathecal battery and of male terminalia appears to have been much the same as in *campanulata* and *alexandri*. Absence of the anterior pair of spermathecae was recorded for 19

specimens (1939 series), of the posterior pair in 160, of both spermathecae of one side in 99, as well as of any one, two or three of the battery. Persistent spermathecae were all normal, all abnormal, any one or more normal and the rest abnormal. An abnormal spermatheca had a rudimentary main axis, a rudimentary secondary axis, one axis or the other lacking, the remaining axis occasionally even hypertrophied, terminal portion of one axis bi- or tri-fid, etc.

No information as to seminal vesicles, testes, testis sacs, maturation or reception of sperm was provided. Reproduction must however be uniparental in a majority of such worms and parthenogenesis can be expected.

The AR is being reached, presumably as also in the past, in three ways; through an A stage, through an R, or directly from the H. Neither A or R morphs would seem to be stable in *levis* judging from paucity of individuals and higher incidence of third and fifth order intermediates. The terminal ARZ morph may well have been present, perhaps even more frequently than in Burmese species.

Pheretima elongata (Perrier) 1872

This species has been carried around the world in the tropics but is unknown, at least under its proper name, in Malaysia where it originated. The H morph, as it has been found elsewhere, is not quadrithecal as long thought but polythecal. Spermathecae are in four groups of 3–5 (or more?), in v–vi, with apertures in two pairs of groups on 5/6–6/7. Incidence of abortion of hearts of xiii (one or both) is unusually high and in a series of 58 worms from Rewa (Central India) reached ca. 41%. Abortion of the ventral trunk in viii-ix may be normal in this species. Sperm undoubtedly are matured by some individuals and have been seen in the spermathecae. Parthenogenesis would not, however, be unexpected.

An A morph has been found commonly in Burma and is the one usually obtained elsewhere. Parthenogenesis is anticipated.

Intermediates all have been of the first order, lacking one or more of the groups of spermathecae. The number in each of the retained groups often has been reduced to one. Incidence of I individuals usually has been low; several only in a series of 612 from Kurnool (South India), only 51 secured during ten years collecting in Burma for several hundreds of the A. The highest incidence, in a series of 45 Sandoway worms, was *ca.* 45%. Spermathecae, in all specimens examined, have been diverticulate and apparently normal.

R and AR morphs, or any indications of trends toward them, have not been found.

Pheretima diffringens (Baird) 1869

This worm has been introduced into many parts of the world, perhaps more than any other *Pheretima*. The original home is uncertain though it may have been in China or Japan.

A biparental H seems to be unknown. An H_p morph can be recognized. No evidence of maturation or reception of sperm has been found in many clitellate worms from India, Burma, China, North America, etc. Spermatogenesis, studied by Cognetti (1925), is so aberrant that *diffringens*, according to Omodeo (1952, p. 259) "e quasi sicuramente partenogenetica."

An A morph is known but only from two worms in a series of 60 from an isolated South India (Kodaikanal) colony. Intermediates evidencing trend towards an A have been almost as rare. Three of 13 specimens from northern Burma (Hpungin Hka) in which seminal chambers had not been differentiated in spermathecal diverticula. Two American worms from Ruston, Louisiana, in which the first two spermathecae were lacking and others were incomplete. Intermediates trending toward R are common. Prostates in these I_3 worms, on one or both sides, are small, restricted to xviii, juvenile, rudimentary or even lacking, in which case the prostatic duct may be more

or less rudimentary. An R morph is unknown.

P. diffringens is present in Japan (Gates, 1938) though as yet unrecognized there by local investigators. Worms described under some half dozen other names may be mostly *diffringens*. If so, morph evolution may be more advanced in Japan than elsewhere.

Summary

In the evolutionary process under consideration, organs essential for biparental reproduction in normally hermaphroditic animals are eliminated enabling recognition of intraspecific morphs. These are:

Primary: H. The present day representative of the ancestral form, with biparental reproduction, from which the other morphs have been derived.

Secondary: H_p. Reproductive system complete but some of the organs, testes, prostates, seminal vesicles, remain juvenile.

A. Without spermathecae. Internal storage of foreign sperm after copulation is impossible. Temporary external storage is improbable as adhesive spermatophores have not been developed.

R. Without male terminalia. Discharge of sperm has become impossible.

AR. Without spermathecae and male terminalia. Storage of foreign sperm and extrusion of own sperm both impossible.

ARZ. Testes as well as all other male organs and spermathecae lacking.

Individuals in which evolution to a more advanced morph has not yet been completed are of five orders: I, intermediate between H and A; I_2, intermediate between H and R; I_3, between R and AR; I_4, between H and AR; I_5, between A and AR. Individuals not in direct lines of evolution, the pseudo-intermediates,

TABLE 4. *Polymorphism in* Pheretima levis

Numbers of individuals of various sorts in collections from eight prefectures of the Ou region, northern Honshu

I_3	A	I	H	I_2	R	I_3	I_4	AR	Prefecture
14	6	15	6	9	19	52	16	158	Aomori
51	26	42	5	7	23	85	47	236	Iwate
75	47	38	9	8	43	134	42	217	Miyagi
32	18	16	3	4	26	103	12	142	Fukushima
46	10	8	3	3	5	33	12	159	Aomori
49	18	14	2	2	6	42	13	144	Akita
85	32	24	3	3	12	78	22	171	Yamagata
83	18	20	2	4	20	79	22	435	Fukushima

I_3 Intermediate between R and AR.
I_5 Intermediate between A and AR.
A, H, R, etc., as in previous tables.

Note 1. Original data, as to external reproductive apertures only, is interpreted as above. More detailed information as to internal organs probably would have shifted a number of worms from A and R columns to an appropriate intermediate column.

Note 2. The worms were from 5 to 15 localities in a prefecture.

Note 3. The internal organ or organs corresponding to the external aperture, without exception, was present according to Ohfuchi (1939, p. 87). Some of the structures thought to be intermediate between "capsulogenous glands" and spermathecae may not have been identified correctly. Some of the supposed male pores probably were pores of genital markings. GM glands and genital markings were present at sites of male terminalis and male pores in two American worms.

Note 4. The types of *irregularis* (Goto & Hatai) 1899, the name used by Ohfuchi, are of an AR morph without GM glands or markings. The type of *levis*, also of an AR morph, has genital markings close to each spermathecal pore, each marking associated with a stalked gland of the sort that enabled identification of American material as *levis*. Types of *irregularis*, insofar as can be determined from the original description, could have been of almost any Japanese species with manicate intestinal caeca. GM glands very probably are not capsulogenous though function is unknown. Hence the noncommittal and general name used herein.

may, in some cases, be mutating back toward an H morph.

Divergences from specific or generic norms in systems other than the reproductive may characterize varying percentages of individuals of a morph, but all such are intentionally omitted in above characterizations, as well as minor deviations in reproductive organs, to permit a standard nomenclature that can be used throughout all earthworm families and also the remainder of the Oligochaeta.

Evolution of reproductive-organ polymorphism has been and presumably still is under way throughout the original range of *Pheretima,* regardless of inter- and intraspecific relationships or possible subgeneric groupings, in a wide variety of soils, in near-sea-level plains and high mountains, in monsoon tropic and temperate zone climates. The same evolution is, or has been, underway in other families of earthworms.

A, probably the most common morph, may be the only one established. An R or an AR may be the only one in other species, an R alone may be lacking. A complete set of morphologically modified secondary morphs is as yet unknown but is anticipated in at least one Japanese species. The AR may evolve from the A, the R, or directly from the H by concurrent elimination of spermathecae and male terminalia, and in a single species in more than one way. Early stages in evolution of polymorphism, prior to establishment of any of the A–Z series, may be shown by H_p morphs in some species, and/or by intermediates. Incidence of any particular morph varies from one locality to another and may be 100% in areas to which a species recently has been introduced.

If geographical races are distinguishable by differences in systems other than the reproductive, there is possible for each such infra-specific taxon a complete set of morphs and for each morph an appropriate class of intermediates. If however, racial evolution and specific distinctions are expressed in the reproductive system, certain morphs will be anatomically indistinguishable from each other.

Morphs may be stable or unstable. Stability, in *anomala,* for both A and R, is indicated by high percentages in local populations and by absence of intermediates evolving toward a more advanced morph. Instability, for both A and R, in *levis,* is indicated by low incidences in all populations studied and by higher percentages of intermediates evolving from both A and R toward AR.

Uniformity in evolution of polymorphism is not to be expected, either in races or species.

Prerequisite to evolution of reproductive-organ polymorphism in *Pheretima,* since externally adhesive spermatophores have not been elaborated, is such change in the customary method of earthworm reproduction as will permit it to be uniparental. Parthenogenesis and autogamy are the options but in *Pheretima,* where escape of sperm from a closed system seems unlikely, the available evidence is for the first alternative. Parthenogenesis may prove to be facultative or obligatory but in this connection also uniformity is not to be expected. Organ defect denial of option between uni- and bi-parental reproduction may make parthenogenesis pseudo-obligatory.

Parthenogenesis may be obligatory, only because of male sterility. This may appear prior to or during morph evolution. Sperm may still be matured, sometimes profusely, in a stable morph or even in intermediates evolving to AR. Sperm may be produced in an R morph in which their extrusion is impossible but, in the same species, may not be matured in an A where all apparatus for ejection of viable male gametes is present and apparently functional. Sterilization of germinal tissues may be gradual. In a usually sterile morph an occasional individual may mature a limited amount of sperm. Male infertility may not involve evolutionary elimination of testes. One sterile morph has more testes than its conspecific male-fertile morphs, indeed more than is

known in any other earthworm, sometimes four pairs more than in any species with biparental reproduction alone. Abortion of testes, during early ontogeny, is assumed for the ARZ morph in which those and all other male organs are unrecognizable in adults. Parthenogenesis is established independently of male sterility but the latter will not be perpetuated in absence of the former.

Since parthenogenesis so frequently is associated with male sterility, the changes that will permit the uniparental reproduction of polymorphism are to be sought in oogenesis. Uniformity presumably is no more to be expected in this connection than in others. Hence automixis (Muldal, 1952, p. 71) or some similar process may be involved when parthenogenesis is facultative and apomixis when it is obligatory. Both processes have been found in diploid earthworms of the family Lumbricidae. Polyploidy, which has been found in several European species of that family, does not have to be assumed in *Pheretima* as parthenogenesis is possible in absence of ploidy and as even-numbered ploidy may characterize forms with biparental reproduction.

Organs may be deleted, as in *Pheretima,* in genera where biparental reproduction seems to be obligatory. Such defective individuals are, and must be, rare except when elaboration of externally adhesive spermatophores takes place. In such cases an A morph retaining obligatory amphimixis may be evolved.

If reversal of morph evolution is possible, one result, indicated by the pseudointermediates, may be an H sufficiently different from the original to be specifically distinct.

The reproductive, of all systems, seems to be the most susceptible to evolutionary change when reproduction is parthenogenetic.

Genetic factors responsible for evolution of the A may be inherited independently of those responsible for the R. Both sets of factors must be capable of operating asymmetrically and unilaterally (or of being so inhibited) as well as, in case of the spermathecal battery, in various ways in adjacent segments.

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NOTES AND COMMENTS

SCHISTIC EVOLUTION

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Three patterns of evolution are recognized by Simpson in his most recent essay on evolution (1953, Major Features of Evolution: 377–393). Those 3 patterns were termed splitting, phyletic evolution, and quantum evolution. The first, splitting, was in 1944 (Tempo and Mode: 199–202) called "speciation." This name was rejected in 1953 because of ambiguity resulting from widespread application of that name to several ideas other than the actual splitting phenomenon. To avoid this ambiguity the simple term "splitting" was adopted.

The change of name was not accompanied by any change of concept. Speciation was defined as an essentially "splitting" phenomenon (p. 380): ". . . speciation is the process of differentiation within populations and the rise of genetic isolation between populations formerly part of the same species. Subsequent divergence is not, strictly speaking, speciation but an aspect of phyletic evolution." Thus defined, "speciation" could be used interchangeably with "splitting," in the special context under consideration, and indeed it was by Simpson in subsequent pages.

The titular substitution of "splitting" for "speciation" in "Major Features" accordingly was not an effective solution to the difficulty of ambiguity, for the frequent and equivalent usage of "speciation" carried the same aura of other meanings as before, despite clear restriction of definition.

The particularly confusing connotation of "speciation" in the present context is transformation in allochrony (by phyletic evolution) in addition to differentiation in synchrony (by "splitting"). A distinction between *'transformation,'* applied to allochronic modification, and *"differentiation,"* applied to synchronic modification, is discussed by Simpson (*op. cit.*, 1953: 384). Simpson meticulously avoided application of the term 'speciation' to both phenomena collectively, limiting it to 'differentiation.' That limitation is not necessarily the most reasonable sequel to admission (Simpson, 1951, EVOLUTION, **5**: 296) that ". . . a distinction cannot be made in practice between 'species' in the basic genetical or evolutionary sense and in the sense of subdivisions in a continuous ancestral descendent line." However desirable a restriction of the term "speciation" to the splitting phenomenon may be, the fact remains that the term has so long been applied to both types of modification that an attempted limitation to either is impractical and leads to confusion. Simpson has wittingly discussed this unfortunate irrepressibility of the "worst or most extreme meaning" of words—a sort of Gresham's Law for words, as he aptly terms it.

The dual nature of speciation in this sense, if admitted to embrace both vertical transformation and horizontal differentiation, as a matter of fact lends itself beautifully to integration with the "splitting" and "phyletic" patterns, for vertical speciation is the initial stage of phyletic evolution, horizontal speciation the initial stage of "splitting." That all patterns of evolution should basically involve speciation is completely consistent with Simpson's concepts.

For effectiveness of communication it is highly desirable to substitute a term for "splitting" that (1) may be readily recognized as an hierarchial equivalent of the terms "phyletic" and "quantum" evolution; (2) that has not been and is not regarded as synonymous with "speciation"; and (3) that is not so commonly used in other senses both biological and non-biological. *Schistic evolution* is here suggested as an appropriate substitute. The term "speciation" may be considered thus to include the processes of both (1) vertical transformation, as the initial phase of phyletic evolution, and (2) horizontal differentiation, as a phase of schistic evolution. These processes may occur at any rate within broad limits, roughly subdivided as bradytelic, horotelic and tachytelic. The peculiar pattern evident in conjunction with tachytely is quantum evolution which is thus based upon an entirely different set of criteria than are phyletic and schistic evolution; the three patterns of evolution are not mutually exclusive.

The question may be raised whether the concept of schistic evolution is not limited to the "speciation" level and is thus perhaps simply a type of speciation (granting application of the latter term to both horizontal and vertical deviation). Again it is a matter of definition of speciation. If the entire process of synchronic differentiation of populations, from feebly divergent demes to cenospecies, is regarded as speciation, then certainly it follows that schistic evolution is but a type of speciation and that no additional term is needed for that type (horizontal or synchronic speciation). If this were true there would be no need for recogni-

tion by Simpson (or anyone else) of the distinctive type of evolutionary pattern he designated as "splitting." Logically, it would seem necessary to regard speciation as (1) synchronic "origin of genetic isolation between two populations (or groups of populations)" *and* the allochronic evolutionary equivalent of the synchronic attainment—and not as (2) "the origin of new characters and their distribution or differentiation among and within populations" (quotations from Simpson, 1953: 379). That such a limitation in this particular duality of definition is essential to consistency of thought has been agreed by many if not most students of evolution (*e.g.* Stebbins, Mayr, Muller and a host of others representing botany and zoology, systematics and genetics). Thus limited, speciation is evidently something quite apart from schistic evolution; the concepts are different and distinctive names are warranted.